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

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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 258 and 250 nm respectively. Method C deals with Area Under Curve measurement, which involves the calculation of integrated value of absorbance with wavelength range between 249-267 nm. The drug follows Beer-Lambert’s law in the concentration range of 10-50 µg/ml in all three methods. Results of analysis were validated statistically and found to be satisfactory. Thus proposed anhydrous solvent i.e. Sodium Carbonate and methods can be successfully applied for estimation of Methotrexate in routine analytical work.

KEYWORDS: Methotrexate, Sodium Carbonate, Zero Order derivative, First order derivative, Area Under Curve method (AUC), UV- spectrophotometer.

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. Methotrexate was originally developed and continues to be used for chemotherapy, with or without combination with other agents. 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\textsuperscript{[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.\textsuperscript{[9]} The method were validated according to ICH guidelines.\textsuperscript{[10,11]}

**MATERIALS AND METHODS**

**materials**

Methotrexate was obtained as sample from Aribindo pharmaceuticals and distilled water and Sodium Carbonate (Anhydrous) were used as a solvent in the study.

**instrument**

A Shimadzu UV-1700 UV/VIS spectrophotometer were used with 1 cm quartz cells /cuvet were used for spectral measurements.

**stock solution**

Accurately weigh about 10 mg of Methotrexate was weighed and transferred to 100 ml volumetric flask, 40 ml of Anhydrous sodium carbonate solution (0.1 N) was added to dissolve completely with vigorous shearing/shaking. Then the volume was make up with the distilled water upto mark.

**Method A: Zero order derivative**

The Zero order derivative spectra at n=0 showed a sharp peak at 258 nm (Fig. 1). The absorbance difference at n=0 (dA/d\lambda) were calculated by the software in instrument which is directly proportional to the concentration of the standard solution in cuvet. The standard drug solution were scanned in the Zero order derivative spectra. A calibration curve was plotted taking absorbance difference (dA/d\lambda) against the concentration of Methotrexate. The coefficient of correlation (r\textsuperscript{2}), slope and intercept values of this method are given in Table 1.

**Method B: First order derivative**

The First order derivative spectra at n=1 showed a sharp peak at 250 nm (Fig. 2). The absorbance difference at n=1 (dA/d\lambda) were calculated by the software in instrument which is directly proportional to the concentration of the standard solution in cuvet. The standard drug solution were scanned in the First order derivative spectra. A calibration curve was plotted
taking absorbance difference (dA/dλ) against the concentration of Methotrexate. The coefficient of correlation (r²), slope and intercept values of this method are given in Table 1.

**Method C: AUC (area under curve)**
The AUC (area under curve) involves the calculation of integrated value of absorbance with respect to between the selected 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 of drug were prepared by pipetting out respective ml of solution and volume make up to mark and scanned in the spectrum mode from the wavelength range 400 nm to 200 nm (Fig. 3) and the calibration curve was plotted as AUC against concentration of Drug. The method was checked by comparing/analyzing the samples with known concentrations.

**ANALYSIS OF TABLET FORMULATION**
For the estimation of Methotrexate in tablet formulation, tablets were weighed and ground into a fine powder. Tablet powder equivalent to 10 mg of Methotrexate weighed and transferred to 100 ml volumetric flask and dissolve in 40 ml of Sodium Carbonate Solution. It was kept for ultra sonification for 15 min, finally the volume was made up to the mark with distilled water and this was then filtered through whatman filter paper to get tablet stock solution. Various dilutions of the tablet solution were prepared and analyzed for six times and concentration were calculated by using calibration curve for the three methods. All the methods were validated according to ICH guidelines. Recovery studies were carried out at three different levels i.e. 80%, 100%, 120% by adding the pure drug (8, 10 and 12 mg respectively) to previously analyzed tablet powdered sample (0.5 mg) as per ICH guidelines and percentage recovery was calculated as shown in Table 3. All the methods were validated for linearity, accuracy and specificity.

**METHOD OF 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 interday and intraday precision. The results are shown in the Table 4.
linearity
A series of volumetric flasks of 10 ml capacity were arranged. To each of these flasks 1, 2, 3, 4, 5 ml of the drug stock solution were added. The volume was made up with distilled water. The absorbance was measured at 258 nm in method A, 250 nm in method B and 249-267 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.

RESULT AND DISCUSSION
The solubility of drug is more in anhydrous solvent i.e. Sodium carbonate and all the methods A, B and C for the estimation of Methotrexate in 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 all the methods (Graph 1). The values of standard deviation were satisfactory low and the recovery studies were close to 100% (Graph 2). 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 eradicate the interference by the excipients present in the dosage form. The AUC method has advantage that it is applicable to be drug which shows the broad spectra without a sharp peak. Hence the three methods can be employed for routine analysis of the drugs in Quality Control, RandD laboratories by using Anhydrous solvent like Sodium carbonate.

It has been proved that the stability of the drug in proposed solvent is up to the mark, it can be evaluated by interday and intraday study and all three methods were developed and validated as per ICH guidelines for estimation of Methotrexate. These methods and solvent were used for estimation of marketed formulation. The method has been evaluated for the linearity, precision, LOD and LOQ in order to ascertain the suitability of the method.

High percentage recovery showed that method was free from interference of excipients used in the formulations. The results of the study indicate that the proposed solvent and three UV spectrophotometric methods of analysis can be used in quality control departments with respect to routine analysis for the assay of the tablet containing Methotrexate.
Table 1: Optical characteristics and parameters

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Method A</th>
<th>Method B</th>
<th>Method C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wavelength (nm) (λ Max)</td>
<td>258</td>
<td>250</td>
<td>249-267</td>
</tr>
<tr>
<td>2</td>
<td>Beer’s – Lambert’s range (µg/ml)</td>
<td>10-50</td>
<td>10-50</td>
<td>10-50</td>
</tr>
<tr>
<td>3</td>
<td>Coefficient of correlation (r²)</td>
<td>0.9920</td>
<td>0.9990</td>
<td>0.9940</td>
</tr>
<tr>
<td>4</td>
<td>Regression equation</td>
<td>Y=0.021x + 0.065</td>
<td>Y=0.001x + 0.000</td>
<td>Y=0.002x + 0.002</td>
</tr>
<tr>
<td>5</td>
<td>Slope (m)</td>
<td>0.021</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>6</td>
<td>Intercept (c)</td>
<td>0.065</td>
<td>0.000</td>
<td>0.002</td>
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<tr>
<td>7</td>
<td>LOD</td>
<td>0.66</td>
<td>12.87</td>
<td>4.29</td>
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<tr>
<td>8</td>
<td>LOQ</td>
<td>0.60</td>
<td>11.70</td>
<td>3.90</td>
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Table 2: assay of the tablet

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Method</th>
<th>Conc. (µg/ml)</th>
<th>Amount found (mg)*</th>
<th>% Mean</th>
<th>S.D.</th>
<th>R.S.D. %</th>
<th>S.E.*</th>
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<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>10</td>
<td>9.977</td>
<td>99.77</td>
<td>0.436</td>
<td>4.381</td>
<td>0.178</td>
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<tr>
<td>2</td>
<td>B</td>
<td>9.868</td>
<td>98.68</td>
<td>0.416</td>
<td>5.033</td>
<td>0.170</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>9.276</td>
<td>92.76</td>
<td>0.390</td>
<td>4.203</td>
<td>0.201</td>
<td></td>
</tr>
</tbody>
</table>

*When n=6 at each level of recovery.

Table 3: Recovery studies

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Tablet Sample</th>
<th>Conc.</th>
<th>% Mean</th>
<th>S.D.</th>
<th>R.S.D. %</th>
<th>S.E.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T1</td>
<td>80</td>
<td>100.12</td>
<td>99.73</td>
<td>99.55</td>
<td>0.49</td>
</tr>
<tr>
<td>2</td>
<td>T1</td>
<td>100</td>
<td>99.60</td>
<td>99.52</td>
<td>99.89</td>
<td>0.25</td>
</tr>
<tr>
<td>3</td>
<td>T1</td>
<td>120</td>
<td>99.30</td>
<td>99.13</td>
<td>98.99</td>
<td>0.27</td>
</tr>
</tbody>
</table>

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

Table 4: Statistical validation for precision.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Component</th>
<th>Mean</th>
<th>S.D.</th>
<th>R.S.D.</th>
<th>S.E.</th>
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<tr>
<td>1</td>
<td>Intra-day</td>
<td>99.07</td>
<td>0.49</td>
<td>0.49</td>
<td>0.20</td>
</tr>
<tr>
<td>2</td>
<td>Inter-day</td>
<td>99.03</td>
<td>0.25</td>
<td>0.25</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Fig. 1 spectrum by zero order derivative method.

Fig. 2 spectrum by first order derivative method.

Fig. 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.

REFERENCES


