International Journal of Trend in Scientific Research and Development (IJTSRD)
International Open Access Journal
ISSN No: 2456 - 6470 | www.ijtsrd.com | Volume - 2 | Issue – 4

# Method Development of Methotrexate in Phosphate Buffer Solution by UV-Visible Spectroscopy

**Nikhil Rajnani, Dr. Nalini Satish Kurup**
Department of Pharmaceutics, University of Mumbai,
Prin. K. M. Kundnani College of Pharmacy, Cuffe Parade, Mumbai, Maharashtra, India

## ABSTRACT
A simple, precise, accurate, cost effective stability indicating UV Spectrophotometric method has been developed for the estimation of Methotrexate shows highest λmax at 303nm. Beer's law (linearity response) was found over a concentration range of 2-10 µg /mL with good correlation coefficient (r2 = 0.9987 and the values of standard deviation were satisfactory low and the recovery studies were close to 100%. The Proposed spectrophotometric method was validated as per the ICH Q1A (R2) guidelines. Hence this method can be safely be employed for the routine quality control analysis of Methotrexate.

***Keywords:*** *Methotrexate, Method Development, UV-Visible Spectroscopy*

## INTRODUCTION
Methotrexate is an antineoplastic antimetabolite, IPUAC name 2S)-2-[(4-{[(2, diaminopteridin-6-1(methyl)](methyl) amino} phenyl) form amide]} is a drug for the treatment of certain types of psoriasis and psoriatic arthritis. It may also be useful for other immune system related inflammatory diseases. The drug acts as a selective inhibitor of the enzyme folic acid reductase and inhibits DNA synthesis and cellular replication. It is abbreviated MTX and it is official in Indian Pharmacopoeia, USP, BP

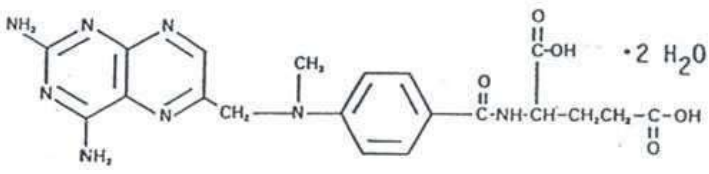

**Figure.1 Structure of Methotrextae**

## MATERIALS AND METHODS

### Materials:

Methotrexate pure drug was procured as gift sample by Neon Laboratories Mumbai. Drug was analyzed on UV Spectrophotometer (Jasco V630)

### Selection of detection wavelength:-

To determine the optimum λmax, Methotrexate 10 µg /mL of working standard solution was prepared in Phosphate Buffer Solution and scanned in UV wavelength range of 200 - 400 nm utilizing as a blank. It was observed that the drug showed maximum absorbance at 303 nm which was chosen as the detection wavelength for the estimation of Methotrexate.

### Preparation of stock and working standard solution:

Methotrexate 10 µg/ mL standard stock solution was done by transferring precisely weighed 10 mg of Methotrexate to 10 ml volumetric flask and dissolved in Phosphate Buffer Solution. The volume was filled up to the mark with Phosphate Buffer Solution. From this solution 1 ml was precisely transferred into 10ml volumetric flask and volume was made up to the mark with Phosphate Buffer Solution. From this solution 1 ml was precisely transferred into 10ml volumetric flask and volume was made up to the mark with Phosphate Buffer Solution to obtain concentration of 10 µg/mL. Figure 2 shows the overlain spectrum of Methotrexate.

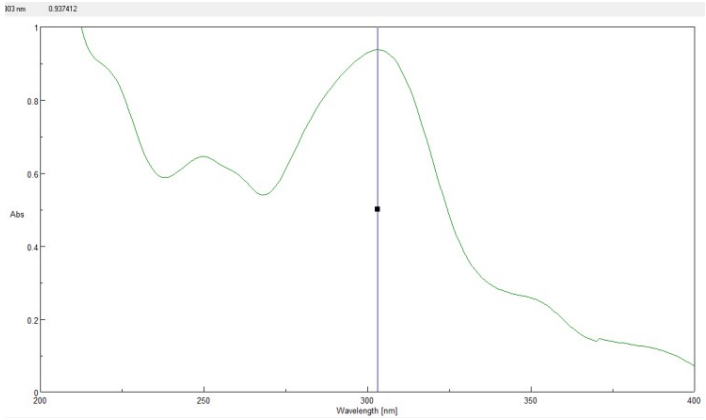


**Figure.1 UV Spectrum of Methotrexate in Phosphate Buffer Solution**

**Preparation of Calibration curve:**

A calibration curve was plotted over a concentration range of 2-10 µg/mL for Methotrexate. Calibration curve was done by plotting Methotrexate concentration on X-axis and their respective absorbance's on Y-axis. Calibration data is shown in Table No. 1. And calibration curve is exhibit in Figure 3.

**Table No. 1 Calibration Data of Methotrexate in Phosphate Buffer Solution**

| Concentration (µg/ml) | Absorbance |
|---|---|
| 2 | 0.2001 |
| 4 | 0.3941 |
| 6 | 0.5786 |
| 8 | 0.7998 |
| 10 | 0.9663 |

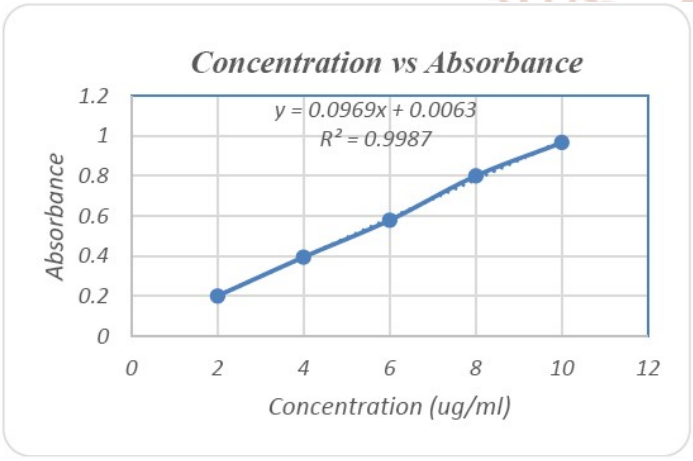


**Figure.2 Calibration Curve of Methotrexate in Phosphate Buffer Solution**

**Precision**

The intra-day precision (repeatability) was evaluated by analyzing six replicates of methotrexate sample solutions (n = 6), at test concentration (10 µg/mL). Similarly, the inter- day precision (reproducibility) was evaluated in two consecutive days (n = 12) and the relative standard deviation (RSD) was calculated. Precision data is shown in the Table No. 2.

**Table No. 2 Precision data**

| Concentration | Intraday | Interday | Standard Deviation |
|---|---|---|---|
| 6 µg/ml | 0.5821 | 0.5884 | 0.01 |
| 8 µg/ml | 0.7895 | 0.7985 | 0.01 |

**Accuracy**

Methotrexate standard solutions, at three different nominal levels (50%, 100% and 150%), At each level, solutions were prepared in triplicate and the recovery percentage was calculated. The mean percentage recovery of methotrexate at each level between 98 and 102% indicated the accuracy of the analytical method. Recovery data is shown in the table no. 3

**Table No. 3 Recovery data**

| Nominal | Theoretical Concentration | Experimental Concentration | % Recovery |
|---|---|---|---|
| 50% | 4.5 µg/ml | 4.41 µg/ml | 98.12 |
| 100% | 6 µg/ml | 6.07 µg/ml | 101.24 |
| 150% | 7.5 µg/ml | 7.51 µg/ml | 100.26 |

**DISCUSSION:**

The UV spectra of Methotrexate was scanned in the region between 200-400 nm. Methotrexate show absorbed maximum at 303nm which was selected as the detection wavelength. The response of the Methotrexate was found to be linear in the ranges from 2-10 µg/mL with a good correlation. The values of standard deviation were satisfactory low and the recovery studies were close to 100%. Optical Parameters are shown in Table No. 4

**Table No. 4 Optical Parameter**

| Parameter | Value |
|---|---|
| Absorbance | 303nm |
| Range | 2-10µg /ml |
| Correlation Coefficient | 0.9987 |
| Regression Equation | 0.0967x+0.0063 |
| % Recovery | 98-101% |
| Limit of Detection | 0.439µg |
| Limit of Quantification | 1.33µg |

## CONCLUSION

Simple, precise and economical UV-visible spectrophotometric method has been developed for the quantitative estimation of Methotrexate in its API form. Method is developed as per the ICH guidelines. The developed method can be used for the quantification of Methotrexate drug substances in routine analysis.

## REFERENCES


1) Oliveira, A. R.; Caland, L. B.; Oliveira, E. G.; Egito, E. S. T.; Pedrosa, M. F. F.; Silva, A. A. Hplc-Dad And Uv-Vis Spectrophotometric Methods For Methotrexate Assay In Different Biodegradable Microparticles. *J. Braz. Chem. Soc.* **2015**, *26* (4), 649–659.

2) Patil, P. D. Y. Auv-Spectrophotometric Determination Of Methotrexate In Tablet Dosage Form Patel Faijal *, Shelke Maroti And Suryawanshi Samrat. **2015**, *5* (4), 641–644.

3) Ramacahandra, B.; N.V.S Naidu; P. Sugun; Kantipudi Rambabu. Validation of Uv – Visible Spectrophotometric Method For The Analysis Of Methotrexate In Pharmaceutical Formulations. *İnternational J. Pharm. Pharm. Sci. Res.* **2013**, *3* (3), 108–114.

4) Ramachandra, B.; N.V.S.Naidu; Rambabu; Kantipudi; P.Sugun. Original Article Validation of Uv – Visible Spectrophotometric Method For The Analysis Of Methotrexate In Pharmaceutical Formulations. *Int. J. Pharm. Pharm. Sci. Res.* **2013**, *3* (3), 108–114.

5) Suryawanshi, S.; Shinde, P.; Thamke, N.; Mohite, M.; Padm, D. Y. Spectrophotometric Determination of Methotrexate In Tablet Dosage Form. **2015**, *2* (6), 153– 155.

6) Cristina Magalhães Santos, M.; Da Costa, V. M.; Pereira, A. D. F.; Silva-Cunha, A.; Ligório Fialho, S.; Pereira Santinho Gomes, A. J.; Gisele, R. D. S. Development And Validation Of Spectrophotometric Method For Determination Of Methotrexate Incorporated Into Plga Implants. *Int. J. Drug Dev. Res.* **2013**, *5* (1), 154–160.

7) Subbarayan S., Karthikeyan V. Analytical method development and validation of layer by layer magnetic nanoparticles of methotrexate and melphalan.world journal of pharmacy and pharmaceutical sciences. 3(3): 1221-1253.

8) Maste M. M., Bhat A. R., Mohite M. and Patil D., Spectroscopic method for estimation of Methotrexate in bulk and tablet dosage form. 2011; 2(2): 47-50.

9) Jaroslaw C., Tomasz G. and Janusz B. methods for methotrexate determination in Macromolecular Conjugates Drug Carrier. Acta poloniae Pharmaceutical and Drug Research, 2012; 69(6): 1342n1346.

10) Alice R. Oliveira, Lilia B. Caland, Edilene G. Oliveira, Eryvaldo S. T. Egito, Matheus F. F. Pedrosa and Arnobio A. Silva Junior, HPLC-DAD and UV-Vis Spectrophotometric Methods for Methotrexate Assay in Different Biodegradable Microparticles, J. Braz. Chem. Soc., 2015; 26(4): 649-659.

11) ICH, Q2A, Text on Validation of Analytical Procedures, International Conference on Harmonization, Geneva, October 1994; 1.

12) ICH, Q2B, Validation of Analytical Procedures: Methodology, International Conference on Harmonization, Geneva, November 1996; 1.


## ACKNOWLEDGEMENT

We would like to thank Neon Laboratories for the gift sample of drug