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Analysis of Pralatrexate Using Simple and Fast Reverse Phase High Performance Liquid Chromatographic Method

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Abstract: *A simple, specific, precise, accurate, and sensitive Reverse Phase High Performance Liquid Chromatographic method has been developed for the determination of Pralatrexate in both pure and pharmaceutical dosage forms. In this method Agilent (4.6×150mm) 5µ column in isocratic mode with mobile phase containing water: methanol (25:75% v/v) was selected. The effluents were monitored at 300 nm and flow rate was fixed as 1.4 ml / min. The retention time was 3.312 min. The linearity was in the range of 20-100 µ g / ml. This method was validated for linearity, precision, limit of detection, limit of quantification and accuracy. Statistical analysis proves that the method is precise, reproducible and selective for the estimation of the pralatrexate drug.*

Keywords: *RP-HPLC, Pralatrexate, Validation.*

I. Introduction

The cancer cells generally have an over expression of reduced folate carrier protein-1 (RTC-1) compared to normal somatic cells. This carrier protein allows the entrance of pralatrexate into the cell. Upon entering the cell, folypoly glutamate synthase FPGS catalyzes the poly glutamination of pralatrexate so that it is retained inside the cell. Once inside, pralatrexate competitively inhibits dihydrofolate reductase (DHFR) and thymidylate synthase.[1, 2] Subsequent depletion of thymidine monophosphate (TMP) occurs so that the cancer cell is unable to synthesize DNA and RNA. As a result, the cancer cell cannot proliferate and is forced to undergo apoptosis. Pralatrexate is more effective against cells that are actively dividing.[3, 4, and 5.]

No analytical methods that have been reported so far for the estimation of Pralatrexate by HPLC method. The objective of the work was to develop simple, accurate, precise and economic RP-HPLC method with lesser run time to estimate the Pralatrexate in bulk and pharmaceutical dosage forms.

II. Materials and methods

The liquid chromatographic system consisted of following components. A Shimadzu HPLC model 2695, UV detector 2487 and Pump, variable wavelength PDA detector and Hamilton syringe (50 µL).
Chromatographic analysis was performed using empower software on an Agilent (4.6×150mm) 5µ column. The mobile phase consisting of water and methanol (25:75% v/v). The optimized chromatographic conditions are summarized in Table 1 and Pralatrexate structure is seen in figure 1.

Preparation of Pralatrexate standard preparation

10 mg of Pralatrexate was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of mobile phase which is used as a diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. Further pipette out 1ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent. The solutions were injected using a 20µl fixed loop in to the chromatographic system at the flow rate of 1.4ml/min and the effluents were monitored at 300nm, chromatograms were recorded. The Pralatrexate was eluted at 3.312min as shown in Fig: 2 The method was extended for the determination of Pralatrexate in pharmaceutical dosage form.

Preparation of Pralatrexate sample preparation

20 tablets of Pralatrexate was powdered and average weight of each tablet calculated. From that 10 mg pralatrexate powder was accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make up the volume to the mark with the same diluent. Further pipette 10ml of the above solution into a 100ml volumetric flask and was diluted up to the mark with diluent. The concentration of the drug in tablet sample solution was calculated by comparing the peak area of standard. The proposed method was validated as per the ICH guidelines.

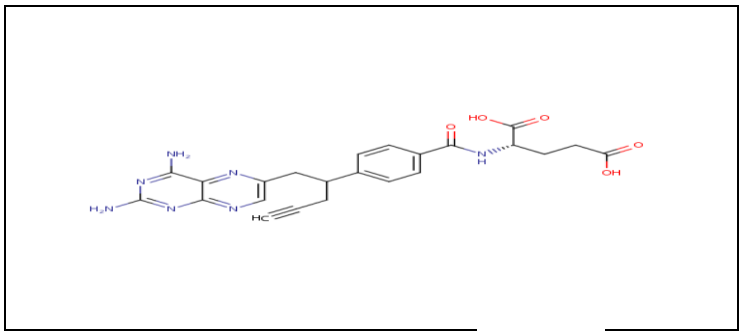

Fig.1: Chemical Structure of Pralatrexate

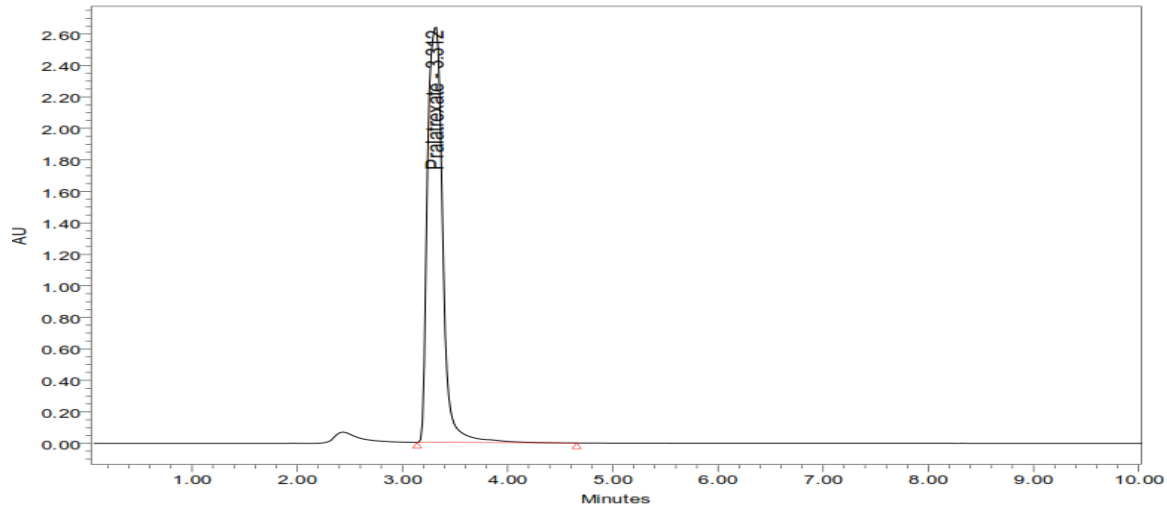

Fig.2: Typical RP-HPLC Chromatogram of Pralatrexate by the proposed method.

Table 1: Optimized Chromatographic conditions for the proposed method

Parameters		**Optimized condition**
Column	:	Agilent (4.6×150mm) 5µ
Mobile phase ratio	:	water: Methanol (25:75% v/v)
Detection wavelength	:	300 nm
Flow rate	:	1.4 ml/min
Injection volume	:	20µl
Column temperature	:	Ambient
Auto sampler temperature	:	Ambient
Run time	:	10min
Retention time	:	3.312min

III. Results and Discussion

The method optimized above was validated and chromatograms of various parameters[6, 7] were obtained. The results obtained were within acceptable limits (Table 2). Thus the system meets suitable criteria. The calibration curve was obtained for a series of concentration in the range of 20-100 μ g/ml and it was found to be linear. The precision was measured in terms of repeatability and intermediate precision was determined by sufficient number of aliquots of a homogenous sample. The % RSD was found and lying within 2. This showed that the precision of the method was satisfactory. The accuracy of the method was inferred from precision and linearity studies of the standard. The % RSD was less than 2.0. This showed that the recoveries of pralatrexate by the proposed methods was satisfactory. Limit of detection (LOD) and Limit of quantification (LOQ) were determined by the proposed methods. The results of validation parameters are summarized in Table 3. The results of recovery studies obtained by the proposed method were evaluated and are given in Table 4. The assay results are mentioned in table 5.

Table 2: System Suitability Test Parameters for the proposed method.

Parameters	**Values**	**Required limits**
Retention time	3.312min	Above 2min
Theoretical plates	3320	N > 2000
Tailing factor	1.2	$T \leq 2$

Table 3: Summary of Validation Parameters for the proposed method

Parameters	**Values**
Limit of detection (μ g/ml)	3.67
Limit of quantification (μ g/ml)	8.87
***Precision (% RSD)**	
Repeatability	1.0
Intermediate precision	0.6

Table.No.4. Showing accuracy results for Pralatrexate

%Concentration (at specification level)	**Average area**	**Amount added (mg)**	**Amount found (mg)**	**% Recovery**	**Mean recovery**
50%	1907860	5	4.86	98.81%	98.96%
100%	3776045	10	9.88	99.08%	
150%	5762457	15	15.0	100.0%	

The accuracy study was performed for % recovery of Pralatrexate. The % recovery was found to be 98.96% (NLT 98% and NMT 102%)

Table 5: Assay Results of Pralatrexate tablets using proposed method

Brand used	**Labelled amount (mg)**	**Amount found (mg)**	**% Recovery**
Injection (folotyn)	20	19.91	99.56%

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