RESEARCH ARTICLE *Rajamani et.al / IJIPSR / 6 (06), 2018, 43-58*
Department of Pharmaceutical Analysis & Quality Assurance *ISSN (online) 2347-2154*
DOI: 10.21276/IJIPSR.2018.06.06.704

International Journal of Innovative Pharmaceutical Sciences and Research
www.ijipsr.com

METHOD DEVELOPMENT AND VALIDATION OF PRALATREXATE BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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Abstract

Method Development and validation of Pralatrexate bulk and pharmaceutical dosage form by RP-HPLC**.** An isocratic reverse phase LC-method was developed using Hypersil ODS C18,150× 4.6mm,5µm column and a mobile phase comprising of a mixture of (ph 3.1±o.1) Acetonitrile And Buffer (30:70v/v).The UV-detector set at 224nm with flow rate of 1ml/min. The method is linear between 30µg ml to 70µg ml with R^2 value as 0.999, the limit of detection (LOD) is 0.452µg ml and limit of quantification (LOQ) IS 1.356µg ml. The accuracy of the method was found to be in the range of 99.86% to 100.98%.The method precision % RSD were less than 2.Stress degradation studies were done for acid, base, H_2O_2 and heat. The proposed method Pralatrexate was found to be linear, precise and accurate for the quantitative estimation of in Tablet and can be used for commercial purposes.

Keywords: Pralatrexate, HPLC, Stress Stability Indicating and Method validation.

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DOI: 10.21276/IJIPSR.2018.06.06.704

INTRODUCTION

Chromatography is a non-destructive procedure for resolving a multi-component mixture of trace, minor, or major constituents into its individual fractions. Different variations may be applied to solids, liquids, and gases. While chromatography may be applied both qualitatively and quantitatively, it is primarily a separation tool. Quantitative analysis can be carried out by measuring the area of the chromatographic peak.

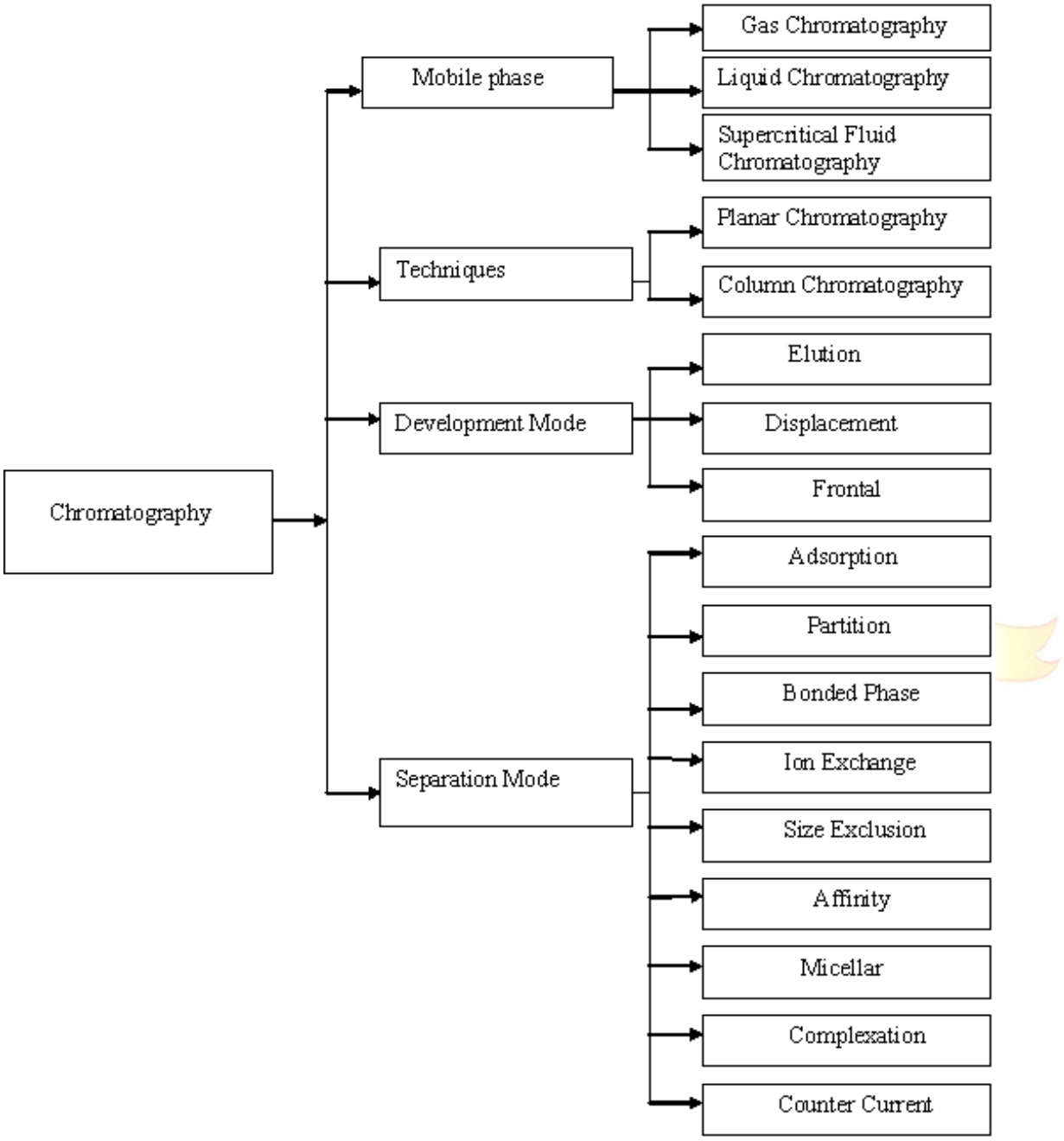

Fig.1: Chromatography Types

DOI: 10.21276/IJIPSR.2018.06.06.704

The separation process is monitored by the integrator from the time of injection to its elution, a graph is obtained. This graph is called a chromatogram. The time required for a compound to elute (called retention time) and the relationship between compound concentration (amount) and peak area depend on the characteristics of the compound. Retention time is therefore used as an index for qualitative determination and peak surface area (or height) as an index for quantitative determination.

There are two elution processes.

- Isocratic elution and
- Gradient elution

MODES OF CHROMATOGRAPHY:

- Normal Phase Chromatography
- Reversed Phase Chromatography
- Reversed Phase – ion pair Chromatography
- Ion Chromatography
- Ion-Exchange Chromatography
- Affinity Chromatography
- Size Exclusion Chromatography

As a general rule the retention increases with increasing contact area between sample molecule and stationary phase i.e. with increasing number of water molecules, which are released during the adsorption of a compound. Branched chain compounds are eluted more rapidly than their corresponding normal isomers.

In reversed phase systems the strong attractive forces between water molecules arising from the 3-dimentional inter molecular hydrogen bonded network, from a structure of water that must be distorted or disrupted when a solute is dissolved. Only higher polar or ionic solutes can interact with the water structure. Non- polar solutes are squeezed out of the mobile phase and are relatively insoluble in it but with the hydrocarbon moieties of the stationary phase.

Chemically bonded octadecylsilane (ODS) an alkaline with 18 carbon atoms it is the most popular stationary phase used in pharmaceutical industry. Since most pharmaceutical compounds are polar and water soluble, the majority of HPLC methods used for quality assurance, decomposition studies, quantitative analysis of both bulk drugs and their formulations use ODS HPLC columns. The solvent strength in reversed phase chromatography is reversed from that of adsorption

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chromatography (silica gel) as stated earlier. Water interacts strongly with silanol groups, so that, adsorption of sample molecules become highly restricted and they are rapidly eluted as a result. Exactly opposite applies in reversed phase system; water cannot wet the non-polar (hydrophobic) alkyl groups such as C_{18} of ODS phase and therefore does not interact with the bonded moiety. Hence water is the weakest solvent of all and gives slowest elution rate. The elution time (retention time) in reversed phase chromatography increases with increasing amount of water in mobile phase [2,3].

BUFFER USED IN REVERSED PHASE

Selection of a buffered aqueous mobile phase for RP-LC may seem intimidating but with an understanding of the fundamental effect of pH[7] on retention of ionic analytes and checking some properties of the buffer options, a logical and reasonable choice can be derived.

The pH of the mobile phase will dramatically affect the retention of ionic analytes within 1.5 pH units of their pKa. Thus, it can be understood why control of mobile phase pH has great utility in method development and is critical for reproducible separations of ionic analytes [4].

Table 1: Buffers

Buffer	pKa (25ºC)	Maximum Buffer Range	UV Cut off (nm)
TFA	0.3	--	210 (0.1%)
Phosphate, p1 $H_2PO_4^{1-}$	2.1	1.1 – 3.1	<200
Phosphate, pK_2 HPO_4^{2-}	7.2	6.2 – 8.2	<200
Phosphate, pK_3 PO_4^{3-}	12.3	11.3 – 13.3	<200
Citrate, pK1 $C_3H_5O(CO_2H)_2(CO_2)^{-1}$	3.1	2.1 – 4.1	230
Citrate, pK2 $C_3H_5O(CO_2H)_1(CO_2)^{-2}$	4.7	3.7 – 5.7	230
Citrate, pK3 C_3H_5O (CO2 -)$_3$	6.4	4.4 – 6.4	230
Carbonate, pK1, HCO_3^{1-}	6.1	5.1 – 7.1	<200
Carbonate, pK_2, CO_3^{2-}	10.3	9.3 – 11.3	>200
Formate	3.8	2.8 – 4.8	210

NORMAL PHASE CHROMATOGRAPHY

In normal phase chromatography, the stationary phase is a polar adsorbent and the mobile phase is generally a mixture of non-aqueous solvents. The silica structure is saturated with silanol groups at the end. These OH groups are statistically disturbed over the whole of the surface. The silanol groups represent the active sites (very polar) in the stationary phase.

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This forms a weak type of bond with any molecule in the vicinity when any of the following interactions are present.

- Dipole-induced dipole,
- Dipole-dipole,
- Hydrogen bonding,
- π-Complex bonding.

These situations arise when the molecule has one or several atoms with lone pair electron or a double bond. The absorption strengths and hence k' values (elution series) increase in the following order. Saturated hydrocarbon < olefins < aromatics < organic halogen compounds < sulphides < ethers< esters < aldehydes and ketones < amines <sulphones< amides < carboxylic acids. The strength of interactions depends not only on the functional groups in the sample molecule but also on steric factors. If a molecule has several functional groups, then the most polar one determines the reaction properties. Chemically modified silica, such as the aminopropyl, cyanopropyl and diol phases is useful alternatives to silica gel as stationary phase in normal phase chromatography. The aminopropyl and cyanopropyl phases provide opportunities for specific interactions between the analyte and the stationary phases and thus offer additional options for the optimizations of separations. Other advantages of bonded phases lie in their increased homogeneity of the phase surface. Resolution with water in weak mobile phase may be most conveniently achieved by drying the solvents and then adding a constant concentration of water or some very polar modifier such as acetic acid or triethylamine (TEA) to the mobile phase. The addition of such polar modifiers serves to deactivate the more polar shape as well as the reproducibility of the retention times. Chromatographic methods can be classified most practically according to the stationary and mobile phases, as shown in the table.2.

Table 2: Classification of Chromatographic methods

Stationary phase	Mobile phase	Method
Solid	Liquid	Adsorption, column, thin-layer, ion exchange, High performance liquid chromatography.
Liquid	Liquid Gas	Partition, column, thin-layer, HPLC, paper chromatography. Gas–Liquid Chromatography.

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Validation of a Stability Indicating Method (SIM)

Method validation can be defined as (ICH) "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".

Method Validation, however, is generally a one-time process performed after the method has been developed to demonstrate that the method is scientifically sound and that it serves the intended analytical purpose.

All the variables of the method should be considered, including sampling procedure, sample preparation, chromatographic separation, and detection and data evaluation. For chromatographic methods used in analytical applications there is more consistency in validation practice with the following key analytical parameters summarised.

Table 3: Data elements required for method validation

Analytical Performance Parameter	Category 1: Assays	Category2: Impurities Quant.	Limit tests	Category 3: Specific tests	Category 4: I.D.
Accuracy	Yes	Yes	*	*	No
Precision	Yes	Yes	No	Yes	No
Specificity	Yes	Yes	Yes	*	Yes
LOD	No	No	Yes	*	No
LOQ	No	Yes	No	*	No
Linearity	Yes	Yes	No	*	No
Range	Yes	Yes	No	*	No
Robustness	Yes	Yes	No	Yes	No

All the variables of the method should be considered, including sampling procedure, sample preparation, chromatographic separation, and detection and data evaluation. For chromatographic methods used in analytical applications there is more consistency in validation practice with key analytical parameters including:

(a) Recovery
(b) Response function
(c) Sensitivity
(d) Precision
(e) Accuracy
(f) Limit of detection
(g) Limit of quantization
(h) Ruggedness
(i) Robustness
(j) Stability
(k) System suitability
(l) Performance test of the method

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DRUG PROFILE

Pralatrexate is an antimetabolite for the treatment of relapsed or refractory peripheral T-cell lymphoma. It is more efficiently retained in cancer cells than methotrexate. FDA approved on September 24, 2009.

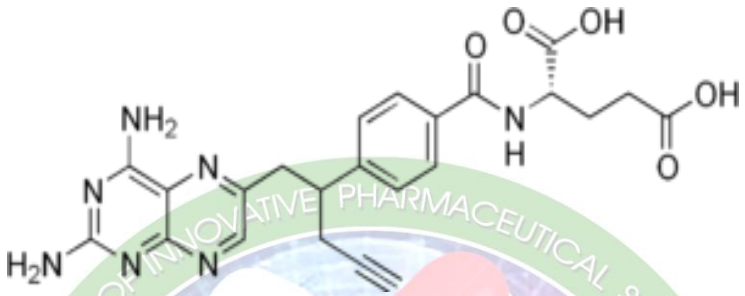

Fig.2: Structure of Pralatrexate

The selectivity of pralatrexate for cancer cells is based upon the observation that 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, folypolyglutamate synthase FPGS catalyzes the polyglutamination of pralatrexate so that it is retained inside the cell. Once inside, pralatrexate competitively inhibits dihydrofolatereductase (DHFR) and thymidylate synthase. 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 [1]

MATERIALS AND METHODS:

Instruments Used

Hitachi L-2130 series consisting pump L-2130,Autosampler,UV-Detector L-2400 Hitachi,Thermostat column compartment connected with Hitachi Elite Lachrome (D-2000Elite)software.

Materials

Active Pharmaceutical ingredient (API) of pralatrexate was obtained as gift samples from comprime Labs(Hyderbad, India).pralatrexate Tablets manufactured by Natco Pharma Ltd were purchased from pharmacy are used in the analysis, its Label claim states that each formulated tablet 40mg and 2ml injection pralatrexate.

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Table 4: Pralatrexate in Folotyn injection

Brand name of Tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Assay % (±SD)
Folotyn	(40mg/2ml)	40.08 (±0.498)	100.2 (±0.343)

METHODS

Mobile Phase Selection

On The Basic of Literature Survey, previous Experience and several exploratory efforts. the chromatographic compatibility was achieved by using p^H 3.1 Acetonitrile :Buffer (30:70) as an Isocratic elution. This gives the results as a mobile phase.

Wavelength selection

After screening the standard solution over 200 to 400 nm wavelength. On the basis of absorption maxima of Analyte 307 nm was decided as maximum wavelength.

Column selection

Column selection is the most important part in the method development. Applying various column Chemistry. The most suitable selected column was Hypersil ODS $C_{18,}$150×4.6mm.5µ

Preparation of mobile phase

Mobile phase was prepared by taking acetonitrile: phosphate buffer (pH 3.1) (70:30 v/v). Mobile phase was filtered through 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min.

Running the standard solution of Pralatrexate

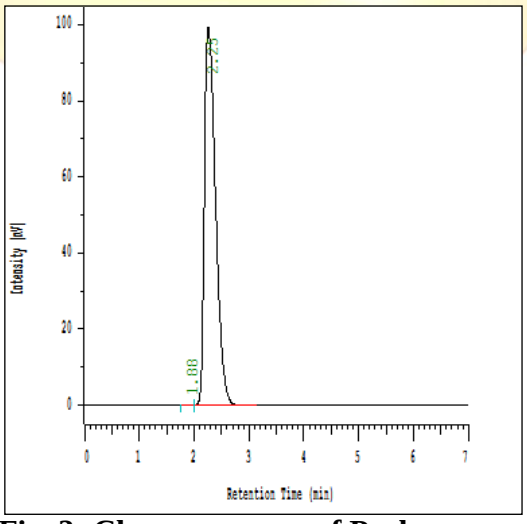

Fig. 3: Chromatogram of Pralatrexate

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Result & Discussion: Retention time was found to be 2.23 min.

Result & Discussion: The HPLC system was set with the optimized chromatographic conditions to run the standard solution of Pralatrexate for 07 min. The retention time were found to be 2.23 min.

FORCED DEGRADATION STUDIES:

Following protocol was strictly adhered to for forced degradation of PralatrexateActive Pharmaceutical Ingredient (API).

The API (Pralatrexate) was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body.

This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after along time storage, within a very short time as compare to the real time or long term stability testing.

The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation and oxidative degradation

1. ACID HYDROLYSIS :

An accurately weighed 25 mg. of pure drug was transferred to a clean & dry 25 ml volumetric flask. To which 0.1 N Hydrochloric acid was added & make up to the mark & kept for 24 hrs. from that 0.2 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of HCl (after all optimized conditions)

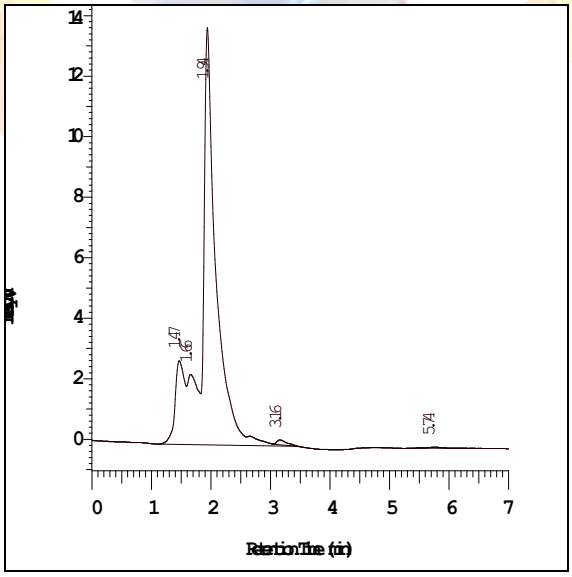

Fig.4: Chromatogram showing degradation for Pralatrexatein 0.1 N HCl

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BASIC HYDROLYSIS

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 0.1 N Sodium hydroxide was added & make up to the mark & kept for 24 hrs. from that 0.2 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of NaOH (after all optimized conditions).

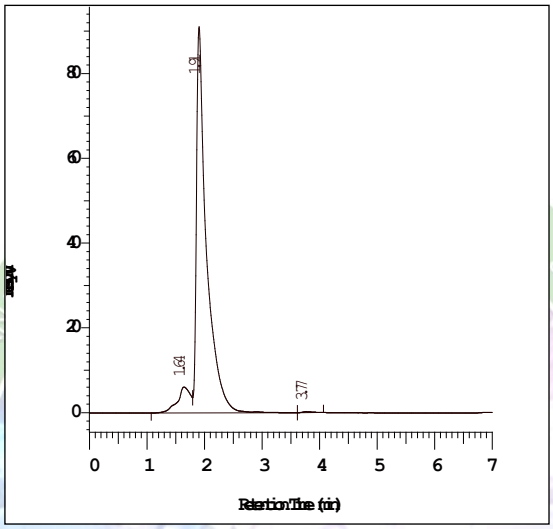

Fig. 5: Chromatogram showing degradation related impurity in 0.1 N NaOH

THERMAL DEGRADATION

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 100 ml volumetric flask, make up to the mark with mobile phase. From this solution take 2 ml make up to the volume 10 ml & was maintained at 50 0C. for 24 hrs. then injected into the HPLC system against a blank of mobile phase (after all optimized conditions)

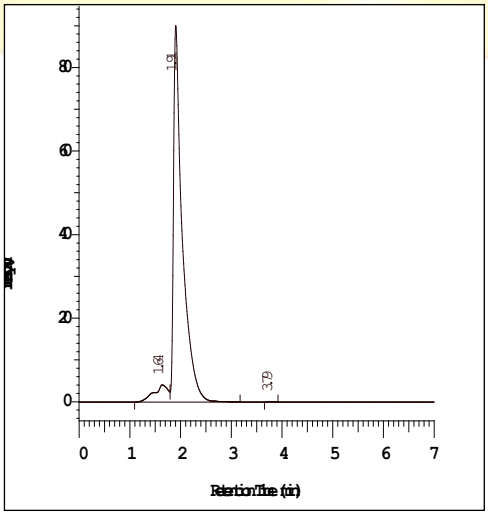

Fig. 6: Chromatogram showing thermal degradation studies

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Photolytic Degradation:

Approximately 10 mg. of pure drug was taken in a clean & dry Petridis. It was kept in a UV cabinet at 307 nm wavelength for 24 hours without interruption. Accurately weighed 1 mg. of the UV exposed drug was transferred to a clean & dry 10 ml. volumetric flask. First the UV exposed drug was dissolved in mobile phase & make up to the mark. Then injected into the HPLC system against a blank of mobile phase (after all optimized conditions)

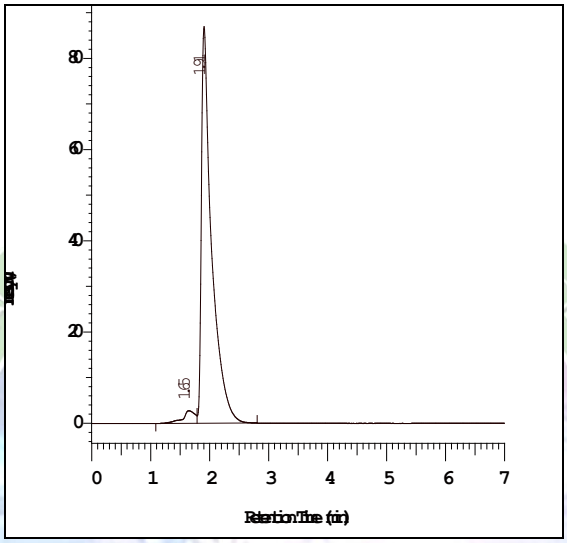

Fig. 7: Chromatogram showing photolytic degradation.

Oxidation with (3%) H_2O_2:

Accurately weighed 10 mg. of pure drug was taken in a clean & dry 100 ml. volumetric flask. 30 ml. of 3% H2O2 and a little methanol was added to it to make it soluble & then kept as such in dark for 24 hours. Final volume was made up to 100 ml. using water to prepare 20 ml solution. The above sample was injected into the HPLC system.

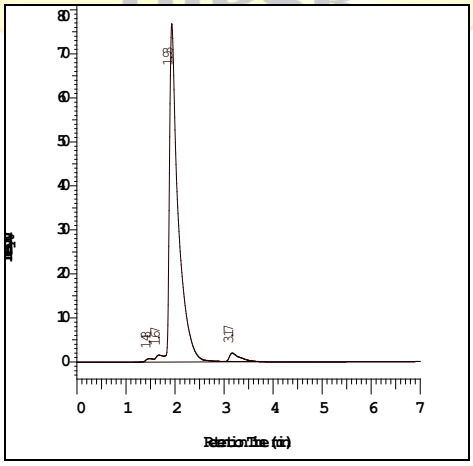

Fig. 8: Chromatogram showing oxidative degradation.

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Results of degradation studies

The results of the stress studies indicated the specificity of the method that has been developed. Pralatrexate was stable only in temperature stress conditions. The result of forced degradation studies are given in the following table.

Table 5: Results of force degradation studies of Pralatrexate API.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	23.75	74.61	98.36
Basic Hydrolysis (0.I MNaOH)	24Hrs.	43.32	55.02	98.32
Thermal Degradation (50 0C)	24Hrs.	97.39	----------	97.39
UV (307nm)	24Hrs.	75.19	24.34	99.53
3 % Hydrogen peroxide	24Hrs.	78.75	20.28	99.03

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Method Validation

Linearity and Range

Linearity range was found to be 0-28 µg/ml for Pralatrexate. The correlation coefficient was found to be 0.995, the slope was found to be 70285 and intercept was found to be 58091 for Pralatrexate.

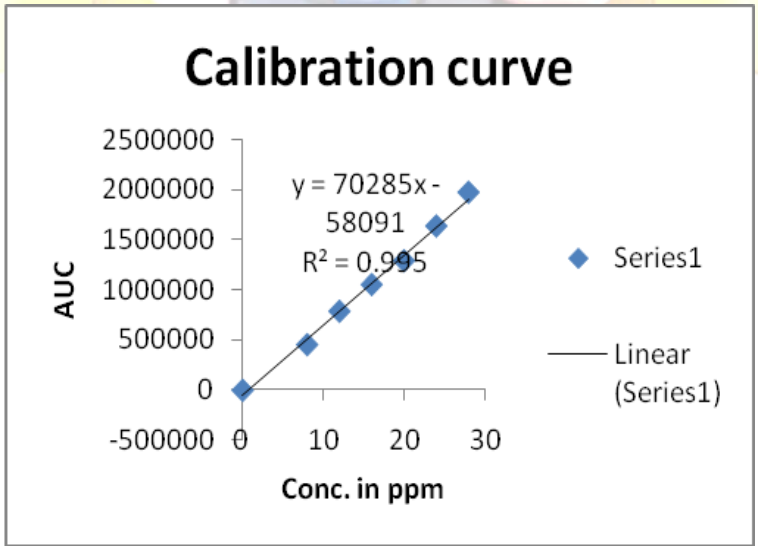

Fig. 9: Standard curve for Pralatrexate

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Table 6 : Standard curve for Pralatrexate

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
8	453700
12	786028
16	1051447
20	1288828
24	1634348
28	1969768

Table 7: Data of recovery studies

ACCURACY			
80% Conc	**peak area**	**Conc. Found**	**% Recovery**
16	1051447	15.78	98.625
16	1072356	16.08	100.5
16	1047630	15.74	98.375
		Avg	99.166667
		SD	1.1614467
		%RSD	1.1712067
100% CONC	**PREAK AREA**	**Conc. Found**	**% Recovery**
20	1288828	19.16	95.8
20	1337202	19.85	99.25
20	1319887	19.6	98
			97.683333
			1.7466635
			1.7880875
120% CONC	**PEAK AREA**	**Conc. Found**	**% Recovery**
24	1684348	24.79	103.29167
24	1679714	24.72	103
24	1675469	24.66	102.75
			103.01389
			0.2711003
			0.2631687

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Result & Discussion

The mean recoveries were found to be 99.16, 97.68, 103.29 % for Pralatrexate. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

Precision

Repeatability

The precision of each method was ascertained separately from the peak areas obtained by actual determination of five replicates of a fixed amount of drug Pralatrexate. The percent relative standard deviations were calculated for Pralatrexateare presented in the table.

Table 8: shows results of repeatability

HPLC Injection Replicates of Pralatrexate	Retention Time	Area
Replicate – 1	2.29	1238828
Replicate – 2	2.31	1237202
Replicate – 3	2.31	1219887
Replicate – 4	2.29	1243249
Replicate – 5	2.31	1215491
Average	2.302	1230931.4
Standard Deviation	0.010954451	12387.3394
% RSD	0.475866688	1.00633873

Result & Discussion: The repeatability study which was conducted on the solution having the concentration of about 20 µg/ml of Pralatrexate showed a RSD of 0.795%. It was concluded that the analytical technique showed good repeatability.

Limit of detection and limit of quantification

The detection limit (LOD) and quantization limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S).

L.O.Q. = 10(SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Result & Discussion

The LOD was found to be 0.452 µg/ml and LOQ was found to be 1.356 µg/ml for Pralatrexate which represents that sensitivity of the method is high.

System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed

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constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table 9.

Table 9: Data of System Suitability Parameter

Sl. No.	Parameter	Limit	Result
1	Resolution	Rs> 2	9.15
2	Asymmetry	T ≤ 2	Pralatrexate = 0.12
3	Theoretical plate	N > 2000	Pralatrexate = 3246

Estimation of Pralatrexate in Tablet Dosage Form

ASSAY:

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak Area of drug obtained with test preparation

AS = Peak Area of drug obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

Result & Discussion: The amount of drugs in Folitrax injection was found to be 40.08 (±0.343) mg/2ml for Pralatrexate & % assay was 99.6 [5,6].

CONCLUSION

A sensitive& selective RP-HPLC method has been developed & validated for the analysis of Pralatrexate API.

Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of Pralatrexate in formulation.

ACKNOWLEDGEMENT

I am very much thankful to St.pauls College of pharmacy, Hyderabad, for giving permission to carry out my work. And I am very much thankful best friend V. Omprakash Chary, A. Mahesh, V. Vinod Kumar.

DOI: 10.21276/IJIPSR.2018.06.06.704

REFERENCES

1. https://www.drugbank.ca/drugs/DB06813.
2. Allos Therapeutics Press Release. (AllosTherapeutics'FOLOTYN(TM) First and only FDA-Approved Therapy for Relapsed or Refractory Peripheral T-cell Lymphoma). Allos Therapeutics, Inc. 2009.
3. Chris H, Takimoto and Emiliano Calvo (Principles of Oncologic Pharmacotherapy). Psychiatric Times, 2005.
4. Robert AN and Alfred HW. Pharmaceutical process validation. 3rd ed., New York, James Swarbrick, 2001.
5. Green J M. A practical guide to analytical method validation. Anal chem., News Feat, 1996.
6. International Federation of Pharmaceutical Manufactures and Associations (IFPMA) Validation of analytical procedures: text and methodology. Proceedings of the International Conference on Harmonization (ICH '96), Methodology Q2 (R1) Geneva, Switzerland. ICH, Switzerland: 1996.