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DEVELOPMENT OF A NEW VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR LEFLUNOMIDE IN BULK DRUG

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Abstract:

A validated stability-indicating RP-HPLC method for Leflunomide (LFN) was developed by separating its degradation products on a Schimadzu C18 (250×4.6mm, 5µm) column using methanol and water in the ratio of 70:30 % v/v as the mobile phase at a flow rate of 1.0 mL/min. The method was validated in terms of specificity, linearity, accuracy, precision, detection limit, quantification limit, and robustness. Forced degradation of Leflunomide was carried out under acidic, basic, peroxide, photo and thermal conditions and the major degradation products of acidic and basic degradation. The linear regression coefficients for Leflunomide were 0.995 in the concentration range 4-12 mcg/ml. The relative standard deviations for intra and inter day precision were 0.75% and 0.33%. The specificity of the method is suitable for a stability indicating assay.

Keywords: *Leflunomide, Method development, Stress testing, RP-HPLC.*

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INTRODUCTION:

According to ICH Q1AR2, SIAM defined as method which is reliable, meaningful and specific means the content of active ingredients, degradation products and other content of interest in a drug product can be accurately measured without interference.

According to US-FDA SIAM, defined as validated quantitative analytical methods can detected the changes with time in the chemical, physical or microbiological properties of the drug substance and drug products, and that are specific so that the contents of active ingredients, degradation products and their components of interest can be accurately measured without interference

Developing and validating new analytical method is costly and time consuming. Before starting the arduous process, a through literature search should be conducted for existing methodologies of the intended analytes or similar compounds. This should include a computerized search of chemical abstract and other relevant source such as compendia monographs (USP, EP), journal articles, manufacturer literature and internet. [1-3]

Leflunomide [Fig.1] is chemically 5-methyl-N-[4-(trifluoromethyl) phenyl]-isoxazole-4-carboxamide. Its molecular formula is C12H9F3N2O2 having molecular weight 270.21g/mole. Leflunomide is a prodrug of the disease-modifying anti-rheumatic drug (DMARD) type, used in active moderate to severe rheumatoid arthritis and psoriatic arthritis. It is a pyrimidine synthesis inhibitor. It is an isoxazole derivative marketed as 10, 20 and 100mg coated tablets [4-7]. Extensive literature survey reveals very few methods have been reported for quantification of leflunomide in bulk drug [8-10]. Reverse phase (RP) HPLC method for determination of pharmaceutical form of drug and also related substances method by RP-HPLC, stability indicating high performance liquid chromatography method. The of present study was to develop a novel simple and economic liquid chromatographic analytical method for leflunomide and to validate the method in accordance with ICH guidelines (ICH Q2 (R), 2006) and to investigate the effect of applying degradative stress to the drug substance[11-12]. Reported work in the literature includes application of limited stress in which a single product seemed to be formed under the action of physical force degradation. In the current work a more intensive stress study was performed on pharmaceutical substance and showed that the drug decomposed into numerous products under different stress conditions. Accordingly, a stability indicating method was established in which the analyte peak was well resolved from those of all the degradation products formed under all stress conditions. The stress study also furnished information about percentage degradation of the drug substance under different stress conditions, information which is not reported in the literature.

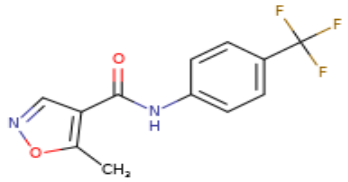


Fig: 1: Chemical structure of Leflunomide

MATERIALS AND METHODS:

Reagents and Chemicals:

Pharmacopoeial grade standard of leflunomide was provided y Hetero drugs Ltd. Analytical HPLC grade solvents Methanol and water were obtained from Spectrochem Pvt. Ltd., Mumbai, India). Analytical grade hydrochloric acid (35%), Sodium hydroxide pellets and hydrogen peroxide solution (30% v/v) were obtained from SD Fine Chem Limited, Mumbai, India.

Instrumentation:

The chromatographic system used to perform development and validation of this method was comprised of a LC-10ATvp Quaternary pump, a SPD-M10Avp photodiode-array detector and auto-sampler model with 10µl loop (Shimadzu, Kyoto, Japan) connected to a multi instrument data acquisition and data processing system.

Chromatographic conditions:

Chromatographic analysis was performed on Schimadzu C18 (250×4.6mm, 5µm) column. The mobile phase was consisted of methanol and water (70:30% v/v). Mobile phase was filtered through a 0.45µm nylon membrane filter (Millipore Pvt. Ltd. Bangalore, India) and degassed in an ultrasonic bath (Spincotech Pvt. Ltd., Mumbai). The flow rate of mobile phase was adjusted to 1.0ml/min and the injection volume was 20µl. Detection was performed at 290nm.

Standard Preparation:

10mg of Leflunomide Standard was accurately weighed and transferred into 100 ml clean dry volumetric flask, and 3/4th volume of Methanol was added mixed well and made up to the final volume with methanol(100µ/ml) . From the above stock

solution, 1ml was pipetted out in to a 10ml volumetric flask and then made up to the final volume with mobile phase and used for further dilutions (10µg/ml). This solution was injected into HPLC system.

Design of the forced degradation study:
The degradation samples were prepared by transferring powdered tablets, equivalent to 10.0 mg Leflunomide into a 250 mL round bottomed flask. Then drug content were employed for acidic, alkaline and oxidant media and also for thermal and photolytic stress conditions. After the degradation treatments were completed, the stress content solutions were allowed to equilibrate to room temperature and diluted with diluent to attain 10 µg/ mL Leflunomide concentrations. Specific degradation conditions were described as follows.

Acidic degradation condition:
Acidic degradation study was performed by refluxed the drug content in 1N hydrochloric acid for 60 min and mixture was neutralized.

Alkali degradation condition:
Alkali degradation study was performed by refluxed the drug content in 1N Sodium hydroxide for 60 min and mixture was neutralized.

Oxidative degradation condition:
Oxidation degradation study was performed by refluxed the drug content in 30% v/v H2O2 for 45 min.

Thermal degradation condition:
Thermal degradation was performed by exposing solid drug to dry heat of 80˚ C in a conventional oven for 3 hr.

Photolytic degradation condition:
Photolytic degradation study was performed by exposing the drug content in sunlight for 72 hr.

RESULTS AND DISCUSSION:

Method development:
The method has been developed by preliminary trials using different composition of mobile phases consisting various mixtures of acetonitrile with phosphate buffer, methanol and phosphate buffer in different ratios, which does not shows proper elution. Finally a mixture of mobile phase consisting of Methanol and Water (70:30v/v) at a flow rate of 1ml/min, shown better elution of peak with satisfying system suitability studies. The column used is Schimadzu C18 (250×4.6mm, 5µm). A detection wavelength of 290 nm was selected after scanning the standard solution over the range 190-400 nm by using photo-diode array (PDA) detector. Detection at 290 nm resulted in good response and good linearity, with a retention time of 4.016min. The method was carried out by standard addition method. After developing the analytical method, it was validated. The analytical method validation gave evidence that the procedure was suitable for the intended purpose; it was carried out as per guidelines of ICH. Typical chromatogram of LFN was shown in Figure 2.

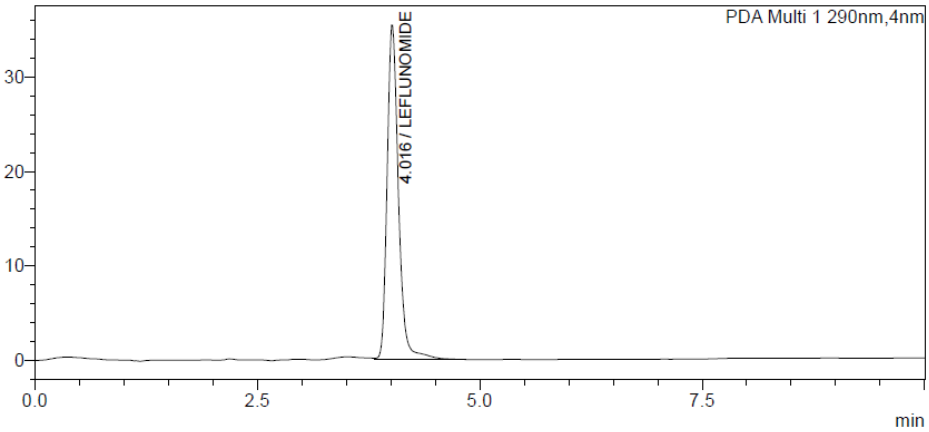


Fig: 2: Chromatogram of Standard Preparation

Validation of the Method:
The developed method has been validated as per ICH guidelines (ICH Q2B) for precision, accuracy, linearity, LOD & LOQ, ruggedness and robustness.

Linearity:
Test solutions for the assay method were prepared at six concentration levels from 40 to 120 % of assay analyte concentration (4, 6, 8, 10 and 12µg/ml) of LFN. The peak areas versus concentration data were evaluated by linear regression analysis. The linearity curve was shown in Figure 3.

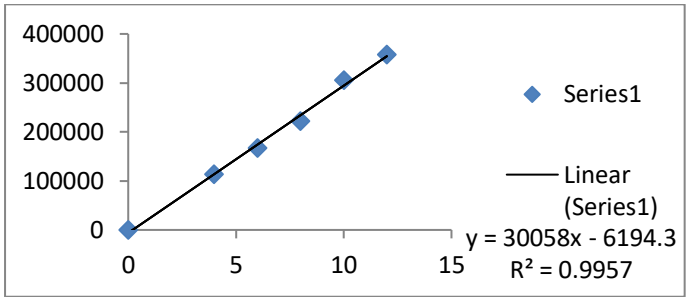


Fig: 3: Linearity curve of Leflunomide

Precision & Intermediate Precision:

The precision of the assay method was evaluated in terms of repeatability by carrying out six independent assays of test sample preparation and calculated the % Relative Standard Deviation (RSD). Intermediate precision of the method was checked by performing same procedure on the different day (interday) by another person under the same experimental condition. The % RSD and assay results are shown in table 1.

Table: 1: Results of Precision studies

S.No	Preparation	Leflunomide
1	Preparation -1	192362
2	Preparation -2	192211
3	Preparation -3	193444
4	Preparation -4	192215
5	Preparation -5	192181
6	Preparation -6	191447
Average	192310	
SD	643	
%RSD	0.33	

Accuracy:

An accuracy study was performed by adding known amounts of LFN to the sample preparation. The actual and measured concentrations were compared. Recovery of the method was evaluated at three different concentration levels (corresponding to 50, 100 and 150 % of test preparation concentration). For each concentration level, three sets were prepared and injected in duplicate. The results were depicted in table 2.

Table: 2: Results for evaluation of accuracy of the method

Level %	No	Amount of drug added (mg/mL)	Amount of drug found (mg/mL)	Recovery (%)	Mean recovery (%)
50	1	0.004020	0.00400	98.20	99.39
	2	0.00400	0.00399	99.90	
	3	0.00400	0.00400	100.08	
100	1	0.00600	0.00599	99.95	99.68
	2	0.00600	0.00598	99.10	
	3	0.00600	0.00600	100.0	
150	1	0.00800	0.00801	100.20	99.78
	2	0.00800	0.00799	99.95	
	3	0.00801	0.00798	99.2	

Robustness:

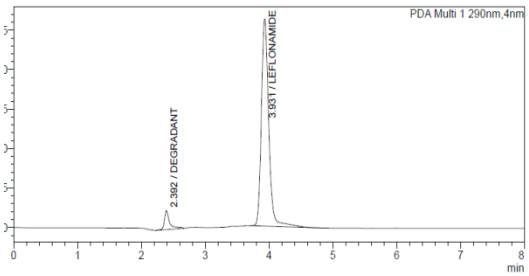
The robustness of study was carried out to evaluate the influence of small but deliberate variations in the chromatographic conditions. The factors chosen for this study were the flow rate (± 0.1ml/min) and Mobile phase compositions (± 10% v/v). The results were shown in table 3.

Table: 3: Results from evaluation of the robustness of the method

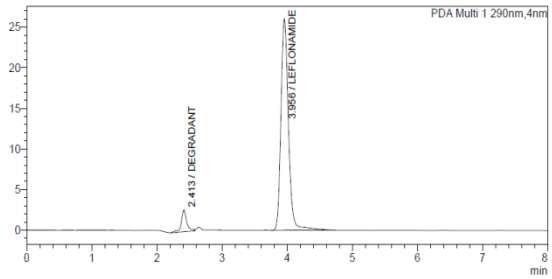
S.No.	Parameter	Leflunomide		
		Rt (min)	Theoretical plate count	Asymmetry
1	Standard	4.016	30083	1.252
2	Change in organic phase ratio (+) 60:40	4.009	31773	1.222
3	Change in organic phase ratio (-) 80:20	4.026	31644	1.223
4	Change in flow rate (-) 0.8ml/ min	4.990	34949	1.277
5	Change in flow rate (+) 1.2 ml/ min.	3.386	26605	1.237

The specificity of the method was determined by checking the interference of blank with analyte and the proposed method were eluted by checking the peak purity of leflunomide during the force degradation study. The peak purity of the leflunomide was found satisfactory (peak purity index (1.0000) under different stress condition. There was no interference of any peak of degradation product with drug peak.

Major degradation of Leflunomide tablets was found with acidic (19.44%) (**Fig. 4a**), alkali (33.54 %) (**Fig. 4b**) and oxidative (13.28 %) (**Fig. 4c**) condition. While they were also degrade under the thermal (0.91%) (**Fig. 4d**) and photolytic (0.33%) (**Fig. 4e**) degradation and results were depicted in table 4. The values obtained were satisfactory and in accordance with guideline limits. The values were show in table 5.



(a)



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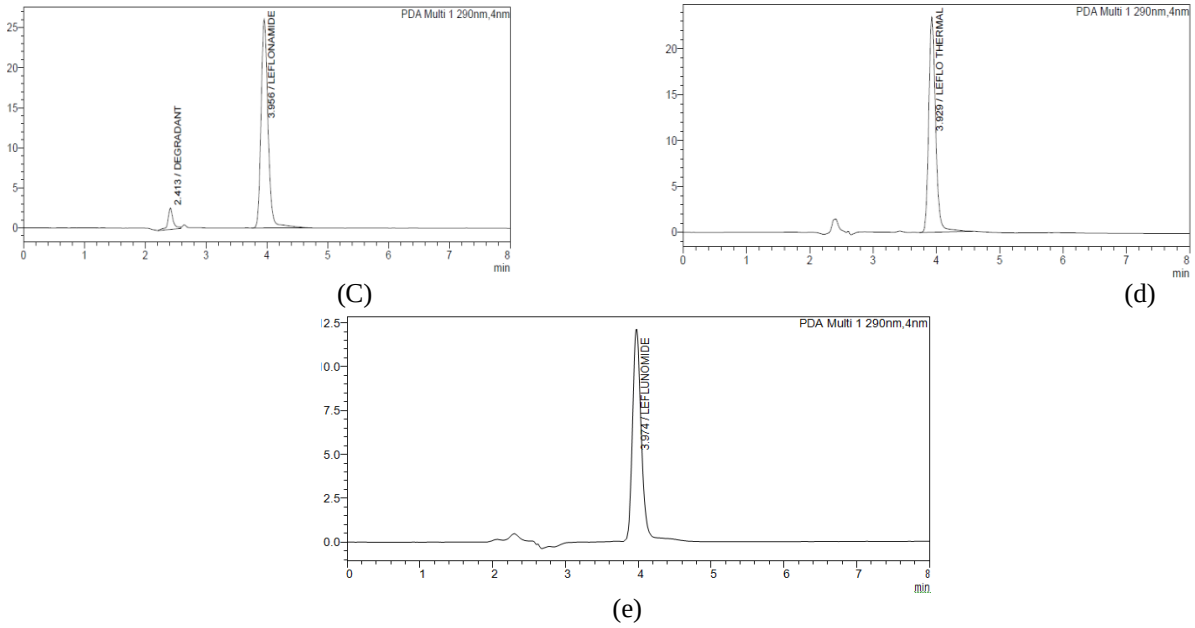

Figure: 4: Chromatogram of: (a) Acidic forced degradation study (b) Alkali force degradation study (c) Oxidative degradation study (d)Thermal degradation (e) Photolytic degradation

Table: 4: Result of Forced degradation studies

S. No	Stress condition	Peak area	% Assay	% Degradation
01	1N HCl	211094	80.60	19.40
02	1N NaOH	176700	60.46	39.54
03	30% H_2O_2	198257	86.72	13.28
04	Thermal	294879	99.09	0.91
05	Photolytic	303897	99.67	0.33

Table: 5: Results from evaluation of the suitability of the method

PARAPMETERS	**Leflunomide**
Linearity	4-12µg/ml
Equation Y=mx+c	y = 30058x - 6194.
Correlation coefficient	0.9995
LOD(µg/ml)	0.65
LOQ(µg/ml)	1.99
system Precision	1.31
method Precision	0.33
Theoretical plates	30083
Peak area	314461
Asymmetry	1.252
Retention time	4.016
% Recovery	99.10%

CONCLUSION:

The RP-HPLC method for determination of Leflunomide in bulk drug was successfully developed and validated for its intended purpose. Sample recoveries using the developed method were in good agreement with their theoretical drug content. The method shown to Specific, linear, precise, accurate and robust. Because the method separates leflunomide and

all the degradation products formed under variety of stress conditions it can be regarded as stability indicating. This method is easily recommended for the routine quality control analysis leflunomide to quantify in pharmaceutical preparations.

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