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Development and validation of a stability indicating UPLC assay method for determination of Leflunomide in tablet formulation

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ABSTRACT

The objective of the current study was to developed simple, precise and accurate isocratic reversed-phase stability indicating Ultra Performance Liquid Chromatography (UPLC) assay method and validated for determination of Leflunomide in solid pharmaceutical dosage forms. Isocratic RP-UPLC separation was achieved on a Water Acquity BEH C18, 2.4 x 50mm, 1.7µ column. The mobile phase was consisted of acetonitrile and 0.02M ammonium acetate buffer (60:40, v/v). at a flow rate of 0.4 ml/min and the detection was carried out at 260 nm by using photo-diode array detector. The flow rate of the mobile phase was adjusted to 0.4 mL/min and the injection volume was 5 µl. The drug was subjected to oxidation, hydrolysis, photolysis and heat to apply stress condition. The method was validated for specificity, linearity, precision, accuracy, robustness and solution stability. The method was linear in the drug concentration range of 10-30 µg/ml with a correlation coefficient 0.9998. The precision Relative standard deviation (RSD) amongst six-sample preparation was 1.16 % for repeatability and the intermediate precision (RSD) amongst six-sample preparation was 0.60 %. The accuracy (recovery) was between 99.44 and 100.24 %. Degradation products produced as a result of stress studies did not interfere with detection of Leflunomide and the assay can thus be considered stability indicating.

Key words: Leflunomide, Stability indicating assay, Method validation, Isocratic.

INTRODUCTION

Leflunomide is a pyrimidine synthesis inhibitor belonging to the DMARD (disease-modifying antirheumatic drug) class of drugs, which are chemically and pharmacologically very heterogeneous [1, 2].

The substance is sold under the brand name Arava by Sanofi-Aventis. It is available for oral administration as tablets containing 10, 20, or 100 mg of active drug. Arava also contains some

inactive ingredients, which could cause allergies or intolerances. Arava was approved by FDA and in many other countries (e.g., Canada, Europe) in 1998.

Leflunomide is chemically 5-Methyl-N-[4(triflurомethyl)phenyl]-isoxazole-4-carboxamide [Figure 1]. Its molecular formula is $C_{12}H_9F_3N_2O_2$ having molecular weight 270.207 g/mole. Leflunomide is a pyrimidine synthesis inhibitor belonging to the DMARD (disease-modifying antirheumatic drug) class of drugs, which are chemically and pharmacologically very heterogeneous.

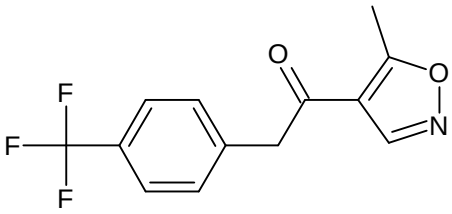

Figure: 1 5-Methyl-N-[4(triflurомethyl)phenyl]-isoxazole-4-carboxamide

Some method have been reported like, UV, TLC, LC-MS and HPLC methods for the determination of leflunomide in pharmaceutical dosage forms or in metabolite and plasma are reported. HPLC methods applied to the determination of leflunomide [3-15] and Some other method have been reported like, derivative Spectrophotometry [16,17] , HPLC [18], HPTLC [19], spectrophotometric method [20], stability indicating HPLC assay [21], for the determination of different drugs in pharmaceutical dosage forms.

So far to our present knowledge, no validated stability indicating UPLC assay method for the determination of leflunomide in pharmaceutical formulation was available in literature. Our work deals with the forced degradation of leflunomide under stress condition like acid hydrolysis, base hydrolysis, and oxidation, thermal and photolytic stress. This work also deals with the validation of the developed method for the assay of leflunomide from its dosage form (tablets). Hence, the method is useful for routine quality control analysis and also for determination of stability.

MATERIALS AND METHODS

Materials

Leflunomide standard was provided by Hetero drugs Ltd. Leflunomide tablets containing 10mg leflunomide and the inactive ingredient used in drug matrix were obtained from market. Analytical grade ammonium acetate was purchased from Sisco Research Pvt. Ltd., Mumbai (India). HPLC grade methanol and water were obtained from Spectrochem Pvt. Ltd., Mumbai (India). Analytical grade hydrochloric acid, glacial acetic acid, sodium hydroxide pellets and 30% v/v hydrogen peroxide solution were obtained from Ranbaxy Fine Chemicals, New Delhi (India).

Instrumentation

The chromatographic system used to perform development and validation of this assay method was comprised of a LC-10ATvp binary pump, a SPD-M10Avp photodiode-array detector and a rheodyne manual injector model 7725i with 20µl loop (Shimadzu, Kyoto, Japan) connected to a multi-instrument data acquisition and data processing system (Class-VP 6.13 SP2, Shimadzu).

Chromatographic conditions

Chromatographic analysis was performed on SGE SS Wakosil (150mm × 4.6mm i.d., C8RS 5 µm particle size) column. The mobile phase was consisted of acetonitrile and 0.02M ammonium acetate buffer (60:40, v/v). The flow rate of the mobile phase was adjusted to 1.0 mL/min and the injection volume was 20 µl. Detection was performed at 260nm.

Standard preparation

Standard solution containing leflunomide (100 µg/mL) was prepared by dissolving accurately about 10.0 mg in 100 mL volumetric flask by diluent [acetonitrile-buffer (50:50, v/v)] (stock standard solution). 10 mL of stock solution was pipetted out into 50 mL volumetric flask and dilute up to mark with diluent (standard solution). The concentration obtain was 20 µg/mL of leflunomide.

Test preparation

Twenty tablets were weighed and the average weight of tablet was determined. From these, five tablets were weighed and transfer into a 500 ml volumetric flask. About 50 ml diluent was added and sonicated for a minimum 30 min. with intermittent shaking.

Then content was brought back to room temperature and diluted to volume with diluent. The sample was filtered through 0.45µm nylon syringe filter. 10 mL of filtrate stock solution was pipetted out into 50 mL volumetric flask and dilute up to mark with diluent The concentration obtained was 20 µg/ml of Lelunomide.

Method Validation

Specificity

The specificity of the method was determined by checking the interference of placebo with analyte and the proposed method was eluted by checking the peak purity of leflunomide during the force degradation study. The peak purity of the leflunomide was found satisfactory (0.9999) under different stress condition. There was no interference of any peak of degradation product with drug peak.

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 20 µg/ mL leflunomide concentrations. Specific degradation conditions were described as follows.

Acidic condition

Acidic degradation study was performed by heating the drug content in 1 N HCl at 60° C for 30 min and mixture was neutralized. In acidic degradation, it was found that around 4 % of the drug degraded.

Alkaline condition

Alkaline degradation study was performed by ambient temperature in 0.005N NaOH for 45 min and mixture was neutralized. In alkali degradation, it was found that around 3-4 % of the drug degraded.

Oxidative condition
Oxidation degradation study was performed by heating the drug content in 30% v/v H2O2 at 80° C for 45 min. Major degradation was found in oxidative condition that product was degraded up to 24 %. The major impurity peaks was found at 1.62 min.

Thermal condition
Thermal degradation was performed by exposing solid drug to dry heat of 80˚ C in a conventional oven for 72 hr. In thermal degradation, it was found that around 0.90% of the drug degraded.

Photolytic condition
Photolytic degradation study was performed by exposing the drug content in UV-light for 72 hours. In photolytic degradation, it was found that around 0.86% of the drug degraded.

Linearity
Linearity test solutions for the assay method were prepared at seven concentration levels from 40 to 160 % of assay analyte concentration. The peak areas versus concentration data were evaluated by linear regression analysis.

Precision
The result of repeatability and intermediate precision study are shown in Table 1. The developed method was found to be precise as the %RSD values for the repeatability and intermediate precision studies were < 0.63 % and < 0.69 %, respectively, which confirm that method was precise.

Accuracy
The HPLC area responses for accuracy determination are depicted in Table 2. The result shown that best recoveries (99.58 - 100.17 %) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

Robustness of method
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 ($\pm$0.1 ml/min), mobile phase composition [methanol-buffer (48: 52 and 52: 48, v/v)], buffer pH ($\pm$0.2 pH) and using different lot of Liquid chromatographic column.

Solution stability
The stability of solution for test preparation was evaluated. The solution was stored at ambient temperature and 2-8º and tested at interval of 12, 24, 36 and 48 h. The responses for the aged solution were evaluated using a freshly prepared standard solution.

RESULTS AND DISCUSSION

Development and optimization of the UPLC method
To develop a rugged and suitable UPLC method for the quantitative determination of Leflunomide, the analytical condition were selected after testing the different parameters such as diluents, buffer, buffer concentration, organic solvent for mobile phase and mobile phase composition and other chromatographic conditions. Our preliminary trials using different composition of mobile phases consisting of water with methanol or acetonitrile, did not give good peak shape. By using 0.02M Ammonium acetate buffer in 1000 ml of buffer and keeping

mobile phase composition as acetonitrile-ammonium acetate buffer (60: 40, v/v), best peak shape was obtained. For the selection of organic constituent of mobile phase, acetonitrile was chosen to reduce the longer retention time and to attain good peak shape. Figure 2 and Figure 3 represent the chromatograms of standard and test preparation respectively.

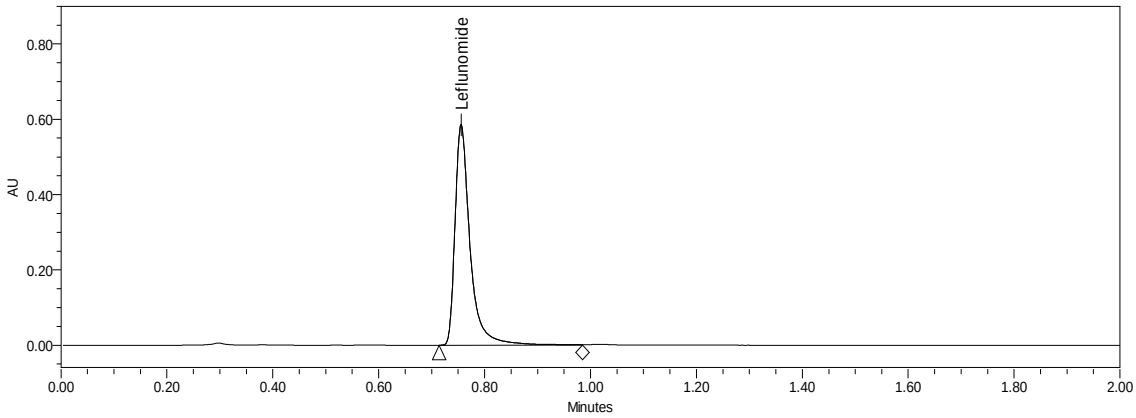

Figure 2: Chromatogram of standard preparation

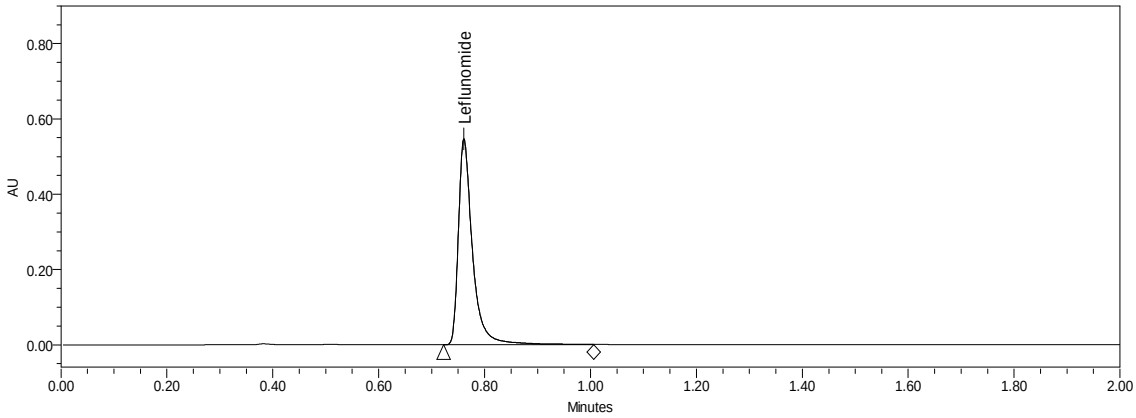

Figure 3: Chromatogram of test preparation

System suitability

A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of standard preparation were injected and asymmetry, theoretical plate and % RSD of peak area were determined for same. Acceptance criteria for system suitability, Asymmetry not more than 2.0, theoretical plate not less then 4000 and % RSD of peak area not more then 2.0, were full fill during all validation parameter.

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 20 µg/ mL leflunomide concentrations. Specific degradation conditions were described as follows.

Acidic condition

Acidic degradation study was performed by heating the drug content in 1 N HCl at 60° C for 2 hours and mixture was neutralized. In acidic degradation, it was found that around 10 % of the drug degraded. (Figure 4)

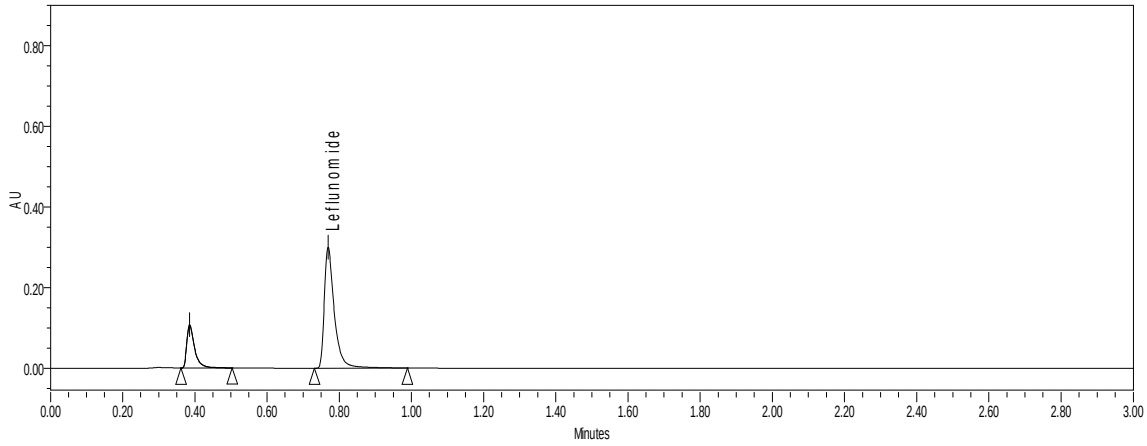

Figure 4: Chromatogram of acidic forced degradation study

Alkaline condition

Alkaline degradation study was performed by ambient temperature in 0.005N NaOH for 2 hours and mixture was neutralized. In alkali degradation, it was found that around 8-9 % of the drug degraded. (Figure 5)

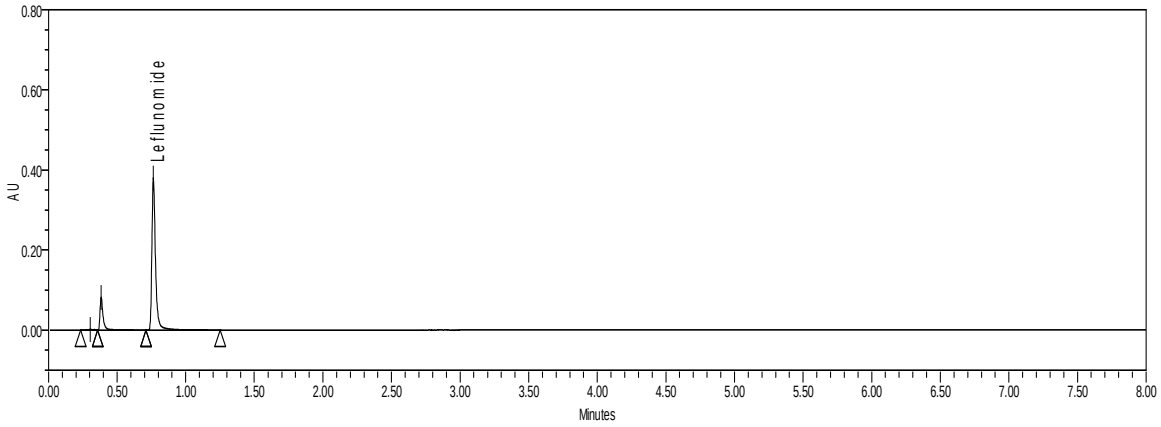

Figure 5: Chromatogram of alkali forced degradation study

Oxidative condition

Oxidation degradation study was performed by heating the drug content in 30% v/v H_2O_2 at 80° C for 45 min. Major degradation was found in oxidative condition that product was degraded up to 22 %. The major impurity peaks was found at 0.5 min. (Figure 6)

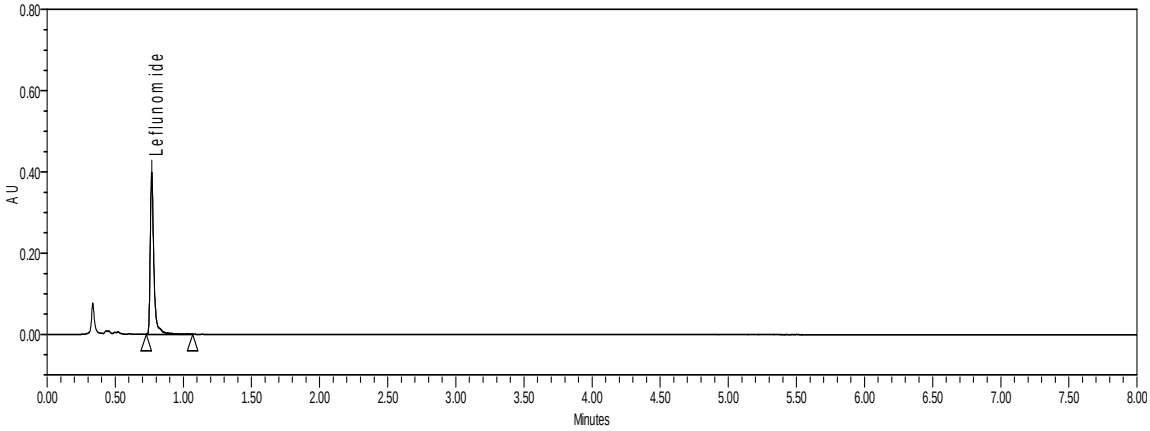

Figure 6: Chromatogram of oxidative forced degradation study

Thermal condition

Thermal degradation was performed by exposing solid drug to dry heat of 80˚ C in a conventional oven for 72 hr. In thermal degradation, it was found that around 0.60% of the drug degraded.

Photolytic condition

Photolytic degradation study was performed by exposing the drug content in UV-light for 72 hours. In photolytic degradation, it was found that around 1.12 % of the drug degraded. (Figure 8)

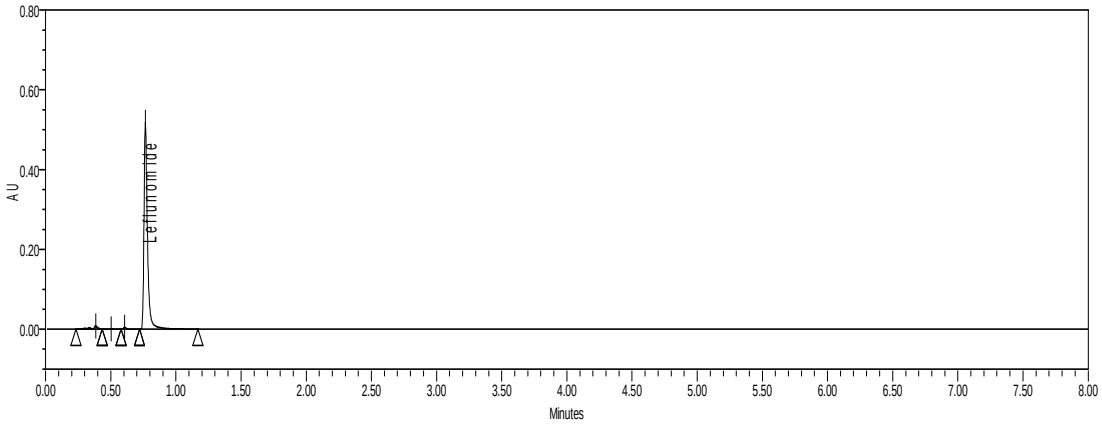

Figure 7: Chromatogram of UV-light degradation study

Linearity

Five points calibration curve were obtained in a concentration range from 10-30 µg/ml for leflunomide. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y=56906771.42857x- 2237.10714 with correlation coefficient 0.9998.

Precision

The result of repeatability and intermediate precision study are shown in Table 1. The developed method was found to be precise as the %RSD values for the repeatability and intermediate precision studies were <1.16 % and < 0.60 %, respectively, which confirm that method was precise.

Table I: Evaluation data of precision study

Set	Intraday (n = 6)	Interday (n = 6)
1	101.8	100.2
2	100.6	99.9
3	101.2	99.5
4	100.4	99.4
5	101.0	98.7
6	98.4	100.3
Mean	100.6	99.7
Standard deviation	1.17	0.60
% [a]RSD	1.16	0.60

[a]*RSD= Relative Standard Deviation*

Accuracy

The UPLC area responses for accuracy determination are depicted in Table 2. The result shown that best recoveries (99.44-100.24 %) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

Table II: Evaluation data of Accuracy study

Level (%)	Amount added concentration [a] (mg/ml)	Amount found concentration [a] (mg/ml)	% Recovery	% RSD
50	0.00987	0.00989	100.24	1.09
100	0.02000	0.01989	99.44	1.24
150	0.02987	0.02978	99.72	1.31

Solution stability

Table III shows the results obtain in the solution stability study at different time intervals for test preparation. It was concluded that the test preparation solution was found stable up to 48 h at 2-5˚ and ambient temperature as during this time the result was not decrease below the minimum percentage.

Table III: Evaluation data of solution stability study

Intervals	% Assay for Test Preparation Solution Stored at 2-8 ˚C	% Assay for Test Preparation Solution Stored at Ambient Temperature
Initial	99.6	99.6
12 h	100.3	100.0
24 h	98.4	98.3
36 h	98.6	98.1
48 h	97.8	97.7

Robustness of method

The result of robustness study of the developed assay method was established in Table 4. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

Table IV: Evaluation data of robustness study

Robust conditions	% Assay	System suitability parameters	
		Theoretical plates	Asymmetry
Flow 0.35 ml/min	99.5	4546	1.45
Flow 0.45 ml/min	102.0	4456	1.20
ACN-Buffer (62: 38,v/v)	98.8	4490	1.35
ACN-Buffer (58: 42,v/v)	98.2	4520	1.58
Column change	99.1	4430	0.58

CONCLUSION

A new analytical method has been developed to be routinely applied to determine Leflunomide in pharmaceutical dosage form. In this study, stability of Leflunomide in present dosage form was established through employment of ICH recommended stress condition. The developed procedure has been evaluated over the specificity, linearity, accuracy, precision and robustness in order to ascertain the stability of the analytical method. It has been proved that it was specific, linear, precise, accurate and robust and stability indicating. Hence, the method is recommended for routine quality control analysis and also stability sample analysis.

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