IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 16, Issue 6 Ser. III (Nov. –Dec. 2021), PP 55-62
www.Iosrjournals.Org

Analytical Method Development and Validation of Leflunomide in Bulk and Pharmaceutical Dosage Form By RP-HPLC Method

IVVALA SAISRI[1], Dr. P.V. MADHAVI LATHA[2]
#1 Master of Pharmacy Scholar, Department of Pharmaceutical Analysis,
#2 Professor, Department of Pharmaceutical Analysis, Viswanadha Institute of Pharmaceutical Sciences, Visakhapatnam, AP, India.

Abstract: *The present study aims to develop an accurate, precise, sensitive, selective, reproducible and rapid analytical technique for the estimation of Leflunomide in bulk and tablet dosage form by using RP-HPLC. To develop a new HPLC method for estimation of Leflunomide and to validate the developed method according to ICH guidelines. To apply the validated method for the estimation of Leflunomide in pharmaceutical formulation A stability indicating method development & validation of Leflunomide was done by RP-HPLC method. The estimation was done by the analysis in RP-HPLC employing Schimadzu C18 (250×4.6mm, 5µm) using mobile phase as Methanol: Water in 70:30 v/v at a flow rate 1ml/min. The linearity range of Leflunomide was found to be HPLC 4-12 µg/ml, with R^2 value of 0.995. The %RSD for intra and inter-day precision was <2%. The % recovery varies in the range of 95-105. The method also passes the specifications for robustness parameters. The results show the method is accurate, precise, sensitive, and economic. The HPLC method is more rapid. Method is successfully applied to the pharmaceutical dosage form.*

Date of Submission: 13-12-2021 Date of Acceptance: 28-12-2021

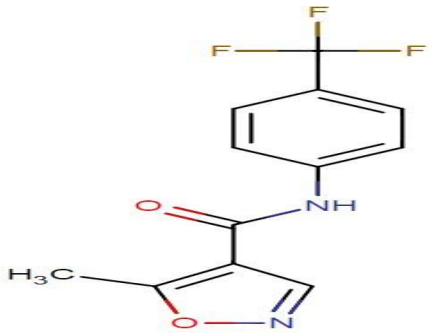
I. Literature Review

MS Palled *et al.,* [23] developed a simple, rapid, reverse phase high performance liquid chromatography (HPLC) method for the estimation of Leflunomide in bulk drug and pharmaceutical dosage form. The separation was achieved with a Hypersil BDS C18 column. This method uses mobile phase consisting of Acetonitrile and 10mM potassium dihydrogen orthophosphate-buffer of PH 4.9±0.1 (90:10) at a flow rate of 1ml/min. Leflunomide was detected by UV-absorption at 254nm with a retention time of 3.03min. The method was carried out by standard addition method. The estimation was linear over the concentration range of 10-50µg/ml, with the correlation coefficient of 0.9999. The intra-day and inter day studies shown that method was accurate and precise, easy-to-operate and validate.

Tapas Kumar Laha *et al.,* [24] developed a simple and economical RP-HPLC method for stability indicating a RP-HPLC method for leflunomide, a disease-modifying anti-rheumatic drug in presence of its degradation of its degradation products formed during forced decomposition studies. Forced degradation studies were performed on the bulk drug by using acid (0.1N hydrochloric acid); base (0.1n sodium hydroxide), water (neutral hydrolysis), 3% v/v hydrogen peroxide (oxidation), dry heat (60^0C) and UV light (254nm). Degradation was observed for leflunomide in acidic and basic media only and the formed degradation products were found to be 5-methylisoxazole-4-carboxylic acid (degradation product-1) and 4(trifluoromethyl)-aniline (degradation product-2). Successful separation of the drug from the degradation products formed under different stress conditions was achieved on a Novapak C18 column (150mm × 3.9 mm, 4µm particle size) using methanol-phosphate buffer pH 5.3; 20mM) (7:3 % v/v) as mobile phase at a flow rate of 1ml/min. the detection wavelength was 260nm. The developed method was completely validated and proved to be robust. As the method could effectively separate the drug from its degradation products, it can be employed for the analysis of samples of the stability study.

II. Drug Profile

LEFLUNOMIDE
Chemical Structure:



Chemical structure of Leflunomide

Chemical name : 5-Methylisoxazole-4-carboxylic acid (4-triflu oromethyl) anilide

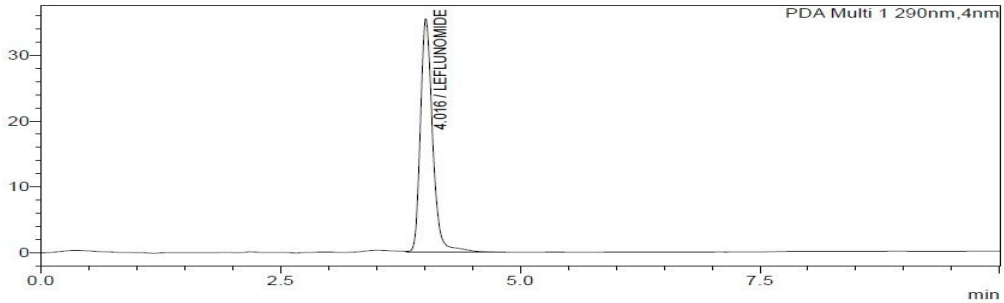
Systematic (IUPAC) name : 5-methyl-N-[4-(trifluoromethyl)phenyl]-1,2-oxazole-4carboxamide

Molecular Formula	:	C12H9F3N2O2
Molecular Weight	:	270.061g/mol.

III. Results And Discussion

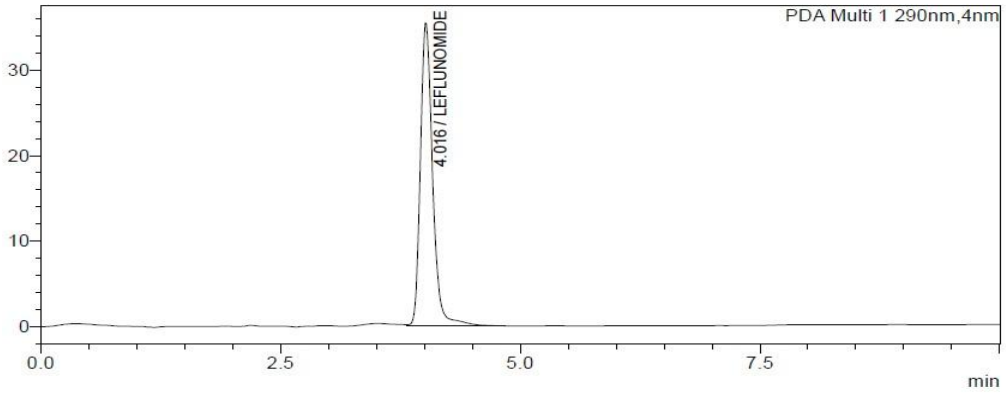
Optimized Method Chromatographic parameters:
Preparation of Mobile Phase:
Prepared mixture of Methanol and Water was taken in the ratio of 70:30% v/v as mobile phase.
Optimized Chromatographic conditions:

Column	:	Schimadzu C18 (250×4.6mm, 5µm)
Mobile phase	:	Methanol: Water (70:30%v/v)
Flow rate	:	1 ml/min
Detection wavelength	:	290 nm
Injection Volume	:	20 µl
Temperature	:	ambient
Run time	:	8min.



Observation: Peak was completely resolved, retention time was less and Peak shape was good.
Discussion: Leflunomide was eluted at 4.016min with good resolution and Asymmetry. Plate count and tailing factor was satisfactory. So this method was considered as optimized and validated.

System suitability:



System Suitability Chromatogram

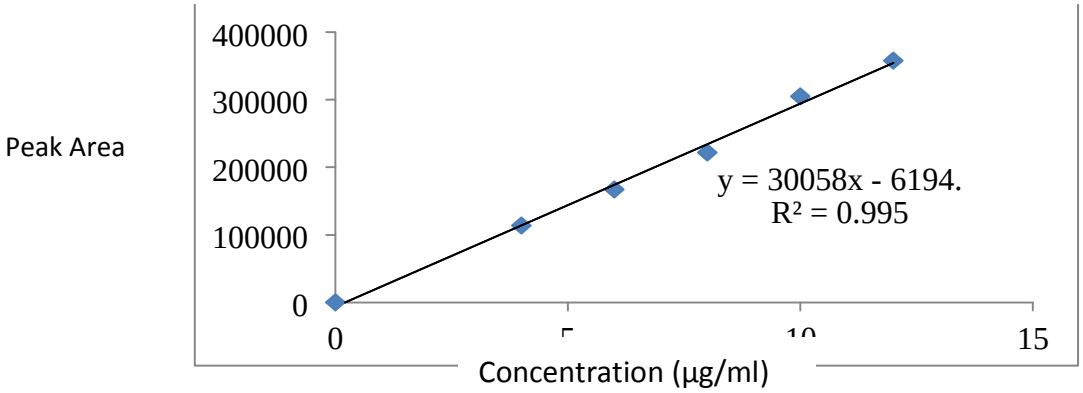
S.No	**Leflunomide**			
Injection	Rt (min)	Theoreticalplates	Peak area	Asymmetry
1	4.016	30083	314461	1.252
2	3.981	30466	314879	1.253
3	4.013	30299	315088	1.195
4	3.99	30099	314761	1.232
5	3.974	30366	313479	1.225
6	4.016	30288	314819	1.252

Results:

PDA:Signal A, 290 nmResults	**Leflunomide**				
Retention time	**Name**	**Area**	**Area %**	**Theoretical plates (USP)**	**Asymmetry**
4.016	Leflunomide	314461	100.00	30083	1.252
Totals		314461	100.00		

Linearity: Linearity data for Leflunomide

Leflunomide	
Concentration (µg/ml)	**Peak area**
4	113740
6	166998
8	221778
10	304879
12	357771
R^2= 0.995	



Discussion: Five linear concentrations of Leflunomide (4-12µg/ml) were injected in a triplicate manner. Average areas were mentioned above and linearity equation obtained for Leflunomide was y = 30058x - 6194, Correlation coefficient was found to be 0.995.

Precision: System precision data of Leflunomide

S.NO	PREPARATION	Area of Leflunomide
1	Preparation -1	194362
2	Preparation -2	194311
3	Preparation -3	190444
4	Preparation -4	197215
5	Preparation -5	192181
6	Preparation -6	196447
Mean	19	4160
SD	2	544
%RSD	1	.31

Discussion: From six different volumetric flasks of standard diluted solutions, six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for Leflunomide pure drug and was found to be 1.31% for Leflunomide.

Method Precision:

Method Precision data of Leflunomide

S.no	Preparation	Area of 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
Mean		192310
SD		643
%RSD		**0.33**

Discussion: From a six different volumetric flask of standard solutions, six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for Leflunomide pure drug and was found to be 0.6% respectively for Leflunomide.

Ruggedness (Intermediate precision): Intermediate Precision (Ruggedness) data of Leflunomide

S.NO	Preparation	Area of Leflunomide

1	Preparation-1	191362
2	Preparation-2	195211
3	Preparation-3	194444
4	Preparation-4	194215
5	Preparation-4	192181
6	Preparation-4	193447
Mean	193476	
SD	1479	
%RSD	**0.75**	

Result:
Results of variability were summarized in the above table. %RSD of peak area was calculated and was found to 0.75.

Accuracy: Accuracy data of Leflunomide

% Concentration (at specification Level)	**Area**			**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% recovery**	**Mean recovery**
	Sample area	**Average**	**Standard area**				
50%	179927	176631	182927	6	5.6	96.6	97.3
	175978						
	170987						
100%	229903	238401		8	7.8	97.5	
	235467						
	249834						
150%	304879	299436		10	9.8	98	

Three levels of accuracy sample were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean % Recovery was obtained as 97.30% for leflunomide.

Sensitivity: Sensitivity data of Leflunomide

Drug	**LOD (µg/ml)**	**LOQ (µg/ml)**
Leflunomide	1.29	3.99

Robustness:

Mobile phase minus (-) chromatogram

PDA:Signal A, 290 nm Results	**Leflunomide**				
Retention Time	**Name**	**Area**	**Area %**	**Theoretical plates (USP)**	**Asymmetry**
4.009	Leflunomide	247327	100.00	31773	1.222
Totals		247327	100.00		

Mobile phase plus (+) chromatogram

PDA:Signal A, 290 nm Results	Leflunomide				
Retention Time	**Name**	**Area**	**Area %**	**Theoretical plates (USP)**	**Asymmetry**
4.026	Leflunomide	247255	100.00	31644	1.223
Totals		247255	100.00		

Flow minus (-) chromatogram

PDA:Signal A, 290 nm Results	Leflunomide				
Retention Time	**Name**	**Area**	**Area %**	**Theoretical plates (USP)**	**Asymmetry**
4.990	Leflunomide	306834	100.00	34949	1.277
Totals		306834	100.00		

Flow plus (+) chromatogram

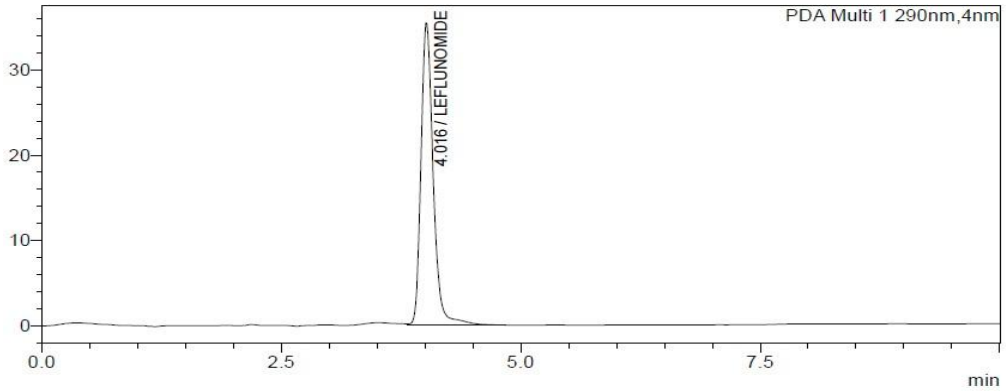
PDA:Signal A, 290 nm Results	Leflunomide				
Retention Time	**Name**	**Area**	**Area %**	**Theoretical plates (USP)**	**Asymmetry**
3.386	Leflunomide	207307	100.00	26605	1.237
Totals		207307	100.00		

Robustness data for Leflunomide

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

Discussion: Robustness conditions like Flow minus (0.8ml/min), Flow plus (1.2ml/min), mobile phase minus (80:20), mobile phase plus (60:40) were maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed and all were found to be within the limits

Assay: % assay of pure drug was found by area normalization method.



Assay chromatogram of Leflunomide

Assay data of Leflunomide

PDA:Signal A, 290 nmResults	**Leflunomide**				
Retention time	**Name**	**Area**	**Area %**	**Theoretical plates (USP)**	**Asymmetry**
4.016	Leflunomide	314461	100.00	30083	1.252
Totals		314461	100.00		

Result: The % assay was found to be 100 %

IV. Conclusion

A simple, sensitive, precise and specific validated RP-HPLC method for estimation of Leflunomide in bulk was developed and validated. The separation was performed on Schimadzu C18 (250×4.6mm, 5µm) chromatographic column. The mobile phase was mixture of Methanol and Water (70: 30). The flow rate was 1.0 mL/ min and detection was performed at 290 nm. According to guidelines, system suitability parameters constitute integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. The developed method was validated according to ICH guidelines. The linear response was observed in the range of 4-12 µg /ml for leflunomide. The percentage recoveries were found to be within limits of acceptance criteria between the ranges of 98 – 102 %. System precision, method precision and intermediate precision were found to be within limits and method was found to be robust. Summary of validation parameters is shown in below table. The method was validated statistically and was applied successfully for estimation of Leflunomide.

Summary table

S. no	**Validation parameter**	**Acceptance criteria**	**Result**		
1	**System suitability**	% RSD for six replicate injections should not be more than 2.0.	0.18		
		The USP plate count for The Leflunomide peak should not less than 2000.	30083		
		The USP tailing for the Lefunomide peak should not more than 2.0.	1.252		
2	**Linearity**	R^2 should be more than 0.995	0.995		
Precision					
3	**System precision**	% RSD for 5 replicate injections should not be more than 2.0 %	1.31		
		The USP plate count for the Leflunomide should not be less than 2000.	30299		
4	**Method Precision**	% RSD for 5 replicate injections should not be more than 2.0 %	0.33		
		The USP plate count for the Leflunomide should not be less than 2000.	31620		
5	**Ruggedness (Intermediate precision)**	% RSD for 5 replicate injections should not be more than 2.0 %	0.75		
		The USP plate count for the Leflunomide should not be less than 2000.	30699		
6	**Accuracy**	The mean % recovery at every level should be 95.0-105.0%	**50%**	**100%**	**150%**
			96.6	97.5	98.0
7	**Robustness**	The system suitability parameters passed for all conditions	The system suitability parameters should passed for all conditions		

CONCLUSION

A stability indicating method development & validation of Leflunomide was done by RP-HPLC method. The estimation was done by the analysis in RP-HPLC employing Schimadzu C18 (250×4.6mm, 5µm) using mobile phase as Methanol: Water in 70:30 v/v at a flow rate 1ml/min. The linearity range of Leflunomide was found to be HPLC 4-12 µg/ml, with R^2 value of 0.995. The %RSD for intra and inter-day precision was <2%. The % recovery varies in the range of 95-105. The method also passes the specifications for robustness parameters. The results show the method is accurate, precise, sensitive, and economic. The HPLC method is more rapid. Method is successfully applied to the pharmaceutical dosage form.

References

[1]. http://www.rxlist.com/crestor-drug.htm. 13/12/2012
[2]. http://www.drugbank.ca/drugs/DB01098. 13/12/2012
[3]. Sweetman, S.C., 2005. Martindale The Complete Drug Reference, 34th Ed. Royal Pharmaceutical Society of Great Britain, 996.
[4]. Lennernas, H., Fager, G., 1997. Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. Clin. Pharmacokinet., 32, 403-425.
[5]. Prabhat, Patel., Ajit, Pandey., Pranita, Kashyap., Indrani, Sahu., 2012. A validated AMD for estimation of Rosuvastatin in bulk and pharmaceutical dosage form by visible spectroscopy. Int. J. Herbal Drug Res., 1(4), 1-4.
[6]. Pushpa, Latha., Uma, Devi., Nagendra, Kumar., Guptha, C. V., Ramalingam, P., 2011. Development and validation of HPTLC method for estimation of Rosuvastatin calcium in bulk and pharmaceutical dosage form. Int. J. Pharma. Bio Sci., 2(2), 134-140.
[7]. Safwan, Ashour., Soulafa, Omar., 2011. Validated HPLC method for the estimation of Rosuvastatin calcium in bulk and pharmaceutical formulations. Int. J. Biomed Sci., 7(4), 283-288.
[8]. Lakshmana, Rao, A., Suneetha, D., 2010. Development and validation of RPHPLC method for the estimation of Rosuvastatin in bulk and pharmaceutical dosage form. Int. J. Chem. Sci., 8(2), 1308-1314.
[9]. Hasumati, A. Raj., Sadhana, J., Rajput., Jayant, B. Dave., Chaggan, N. Patel., 2009. Development and validation of two chromatographic stability-indicating methods for determination of Rosuvastatin in pure form and pharmaceutical preparation. Int. J. ChemTech Res., 1(3), 677-689.

IVVALA SAISRI, et. al. "Analytical Method Development and Validation of Leflunomide in Bulk and Pharmaceutical Dosage Form By RP-HPLC Method." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 16(6), (2021): pp. 55-62.