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Research

Experimental design approach by using rhplc for the determination of rufinamide assay in dose proportional tablet Rufinamide tablets 200 mg

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Abstract

A rapid high performance liquid dosage form. A Kromasil C118, 125 X 4.0 mm, 5µm or or equivalent in isocratic mode, with mobile phase containing a mixture of Weight and transfer about 2.72 of Potassium Di hydrogen phosphate into a beaker containing 1000ml of water, and filter through 0.45µ nylon membrane filter, mobile phase Thoroughly mix buffer, methanol and tetrahydrofuran in the ratio of 80: 15:05 % v/v and Prepare a degasses mixture of water, methanol and acetonitrile in the ratio of 10:50:40 % v/v, The mobile phase was pumped at a flow rate of 1.0 ml/min and the eluents were monitored at 210 nm. The selected chromatographic conditions were found to effectively separator (Rt: 8.98 min) having a resolution of 5.87. The method was validated in terms of linearity, accuracy, precision, specificity, limit of detection and limit of quantitation. Linearity for were found okay respectively. The percentage recoveries for ranged from 99.97-100.0%, respectively. The limit of detection and the limit of quantitation for was found to be 1.5 µg/ml and 3.0 µg/ml, respectively. The method was found to be robust and can be successfully used to determine the drug content of marketed formulations. The method gives resolution with a short analysis time (< 30 min). The method parameter was validated and establish to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the planned method can be used for routine analysis of in medical dosage form.

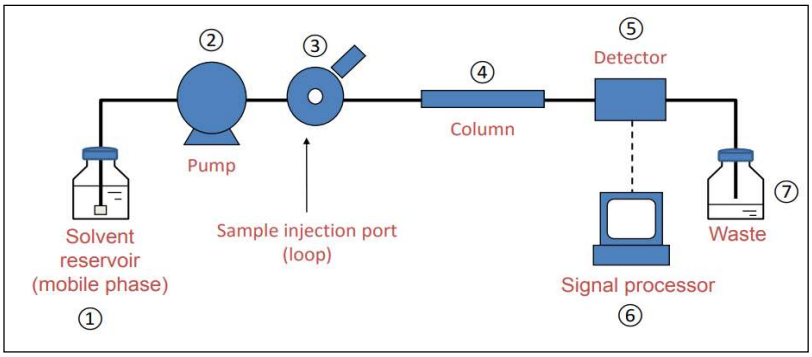
Keywords: Experimental Design, RHPLC, Rufinamide, Formulation, Validation.

INTRODUCTION

HPLC is incredibly versatile, especially when combined with different detectors, such as UV-Visible spectroscopy (UV-Vis), mass spectrometry (MS), and fluorescence. For example, UV-Vis can help determine the concentration of molecules following elution from the column. A highly specific detection method is MS, which

can help measure a molecule's mass-to-charge-ratio and thus its molecular weight. Fluorescence can be particularly sensitive for the right kinds of analytes with parts per billion (ppb) detection limits.

HPLC Block Diagram



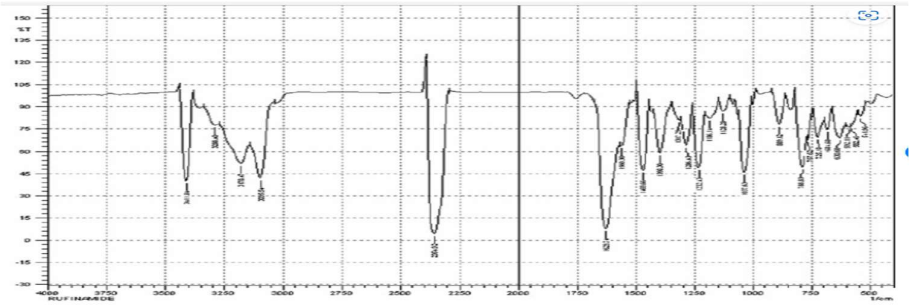
Rufinamide drug

Rufinamide is one of the anti-seizure drugs (ASD) used as an add-on therapy to treat seizures associated with Lennox Gastaut syndrome, and it is structurally unrelated to other traditional ASDs. This CME activity provides a brief and illustrative review of this drug's FDA-approved indications and pharmacodynamic and pharmacokinetic properties. This activity will also highlight the side effects, contraindications, and dosing regimens of this drug and will also discuss the practice parameters of the American Academy of Neurology guidelines regarding the utility of this drug in clinical practice.

Molecular formula and structure: $C_{10}H_8F_2N_4O$1-(2,6-Difluorobenzyl)-1H-1,2,3-triazole-4-carboxamide.

Experimental design approach by using R HPLC for the determination of Rufinamide assay in dose proportional tablet Rufinamide tablets 200 mg.

The present scope is to: Development and Validation of HPLC method for the estimation of assay in tablets 200 mg.The proposed method shall be used for the quantification of active material. The proposed method shall be validated for Specificity, System suitability, Linearity, Accuracy, Range, Precision, and Repeatability and Robustness as per ICH guideline.



IR spectrum for standard Rf.

MATERIALS AND METHODS

Water, Potassium Di hydrogen phosphate, Ortho Phosphoric acid, Trtrahydrofuran, Methanol, Acetonitrile, Sodium Hydroxide, Hydrochloric acid, Hydrogen Peroxide, Rufinamide, HPLC, Analytical Balance, pH Meter, Column, Detector, 0.45 nylon membrane filter, Glassware, Placebo tablets IP 80 mg.

Specificity and System Suitability: Identification, Blank interference of the experiment, System Suitability, Linearity and Range, Precision, System Precision, Method Precision, Accuracy, Solution Stability, System Stability, Solution stability, Robustness, Change in wave length Filter variability.

Chromatographic conditions: Column: Kromasil C18, 125 X 4.0 mm, 5µm or equivalent

Wavelength: UV 210n m, Flow rate Injection: 1.0ml/minute, Volume: 20µLColumn oven Temperature: 30°C Run time: 30 minute

Preparation of the standard solution: Accurately weigh and transfer about 48 mg of standard into a 100 ml volumetric flask add about 10ml of methanol and dilute the volume with diluent and mix. Transfer 5.0 ml of the add solution into a 50 ml volumetric flask. Dilute to volume with dilute and mix

Preparation of Test Sample solution: Weight and powder 20 tablets and transfer about 400 mg of standard into a 100 ml volumetric flask add about 20ml of methanol and sonicate to dissolve and dilute to volume with diluent. Transfer 3 ml of this solution to100 ml volumetric flask and dilute the volume with diluent and mix.

Procedure: Equilibrate the column for not less than 30 minute with mobile phase at flow rate of 1.0ml/minute. Inject 10 µL of blank solution into the Chromatographic system, record the Chromatogram.Program the data processor to inhibit the integration of peaks due to blank. Inject 0 µL of Reference solution into the Chromatographic system, record the Chromatogram and measure the peak response. Inject 10 µL of test sample ample solution into the Chromatographic system, record the Chromatogram and measure the peak response. Inject 10 µL of Reference solution into the Chromatographic system, record the Chromatogram and measure the peak response. (Bracketing standard). Inject Bracketing standard after every 4 samples analysis and/or at the end of the sequence.

Evaluation of System Suitability parameters: The column efficiency as determined for the from standard solution is not less than 3000 theoretical plates and tailing factor for the same peak is not more than 2.0
The relative standard deviation for peak area obtained from five replicate injections of standard solution is not more 2.0

Specificity and System suitability

- **Specificity** is the ability of the analytical method to distinguish between the analyte(s) and the other components in the sample matrix. In case of an HPLC method, it is assured by complete separation of peak(s) of analyte(s) from other peaks originated from the sample matrix.
- **The System Suitability Testing (SST)** is used to verify that an analytical method was suitable for its intended purpose the day the analysis was done. It is an essential parameter to ensure the quality of the method for correct measurements.

Sr.No	Validation Param	Results		Acceptance Criteria
Method and Procedure				
1.	**Identification**	Prepared standard and sample solution as per the test method and inject into the chromatographic system		
2.	**Blank and Placeb Interference**	Prepared blank and placebo solution as per the test method and inject into the chromatographic system		
3.	**System Suitability**	Prepared standard as per the test method and inject into the chromatographic system		
4.	**System Suitability**	**Acceptance Criteria**		
5.	**Identification and RT Confirmation :** The retention time of standard solution and sample solution should be comparable with respect to retention time **Blank and Placebo Interference :** There should not be any interfering peak in the chromatogram obtained from blank solution and placebo solution at the retention time of analyte peak in the chromatogram obtained with the standard **System Suitability** - The column efficiency as determined for the from standard solution is not less than 3000 theoretical plates. - Tailing factor for the same peak is not more than 2 - The relative standard deviation for peak area obtained from five replicate injections of standard solution is not more 2.			
Observed Value				
6.	Identification and I Confirmation	Name	Retention time in minute	The retention time of standard solution and sample solution should be comparable with respect to retention time
		Standard	5.840	
		Sample 20 mg	5.819	
Conclusion: RT of obtained with standard and test sample are comparable. Hence method is specific				

Sr.No	Validation Param	Results		Acceptance Criteria
7.	**Blank and Placebo Interference**	There are no interference peak observed due to place to at the retention time of peak. Hence method is specific		There should not be any interfering peak in the chromatogram obtained from blank solution and placebo solution at the retention time of analyte peak in the chromatogram obtained with the standard
8.	System Suitability	Theoretical plate	Observed value 366.08	The column efficiency as determined for the from standard solution ia not less than 3000 theoretical plates
		Tailing Factor	1.40	Tailing factor for the same peak is not more than 2.
		Retention time	5.885	The relative standard deviation for peak area obtained from five replicate injections of standard solution is not more 2.0
		Deviation	0.02	

System Suitability				
S.No	**Retention Time**	**Peak Area**	**Theoretical Plates**	**Asymmetry**
1.	5.840	2478665	3711.42	1.04
2.	5.819	2490412	3724.88	1.04
3.	5.840	2401567	3716.44	1.04
4.	5.819	2479371	3684.70	1.04
5.	5.819	284130	3735.40	1.04
6.	5.851	2478665	3395.08	1.04
7.	5.819	2490412	3395.08	1.04
8.	5.819	201567	3773.90	1.04
9.	5.851	2489371	3759.19	1.04
10.	5.819	2488130	3691.08	1.04
Mean	5.30			
SD	0.0289			
% RSD	0.36			

Results

Sr.No	Validation Parameter	Results		Acceptance Criteria
Observed Values:	System Suitability	Theoretical plate	Observed value 3669.08	The column efficiency as determined for the from standard solution is not less than 3000 theoretical plates
		Tailing Factor	1.04	Tailing factor for the same peak is not more than 2.
		Retention time	5.819	The relative standard deviation for peak area obtained from five replicate injections of standard solution is not more 2.0
		Deviation	0.13	

Conclusion :

- The retention time of standard solution and sample solution is comparable with respect to retention tim
- There is no any interfering peak in the chromatogram obtained from blank solution and placebo solutic the retention time of analyte peak in the chromatogram obtained with the standard
- The column efficiency as determined for the from standard solution is not less than 3000 theoretical pl
- Tailing factor for the same peak is not more than 2.
- The relative standard deviation for peak area obtained from five replicate injections of standard solution is not more 2.0

Linearity

Linearity of a method is its ability to obtain test results that are directly proportional to the sample concentration over a given range. For HPLC methods, the linear relationship between detector response (peak area and height) and sample concentration is determined. The relationship can be demonstrated directly on drug substance by dilution of standard stock or by separate weighing of the sample components, using the proposed procedures.

Sr.No	Validation Parameter	Results		Acceptance Criteria	
Method and Procedure					
1.	**Method**	Five linearity solutions were prepared by using standard at concentration levels ranging from 50% to 150 % of target concentration of . Measured the peak area response of solution at Level 1 and Level 5 six times and other levels			
2.	**Acceptance criteria**	• **Linearity :** • The co-relation is not less than 0.999 • The % Y intercept is between -2 % to +2 % • % RSD of peak Reponses of 50 % level and 150% level should be NMT 2.0			
3.	Observed results	Correlation Coefficient	0.99995	Correlation coefficient should be not less than 0.999	
		%y-intercept	1.78	%y-intercept should be ±2.0	
		% RSD at lower level	0.07	% RSD of peak area response of 6 replicates at lower and higher levels should be more than 2.0	
		% RSD at higher level	0.18		
Linearity level	**Concentration in ppm**	**Area-Average**	**% of RSD**	**Statistic Analysis**	
L1 (50%)	20.20	1386885	0.18	R^2	0.9999
L2 (80%)	30.180	1037473	--	Slope	62997.06
L3 (100%)	40.240	2614837	--	Y Intercept	42388.40
L4 (120%)	50.300	3564963	---	% Y Intercept	1.78
L5 (150%)	60.360	35911592	0.07	Correlation coefficient	0.99999
				Residual sum of squares	19805
Conclusion : Response of is linear over the concentration range 50% to 150% target concentration					

Precision

Precision of an analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Sr.No	Validation Parameter	Results		Acceptance Criteria
A. System Suitability : Method and Procedure				
1.	**System Suitabil**	Prepared standard solution as per the test methods and inject five times into the chromatographic system		
	Acceptance crit	• The column efficiency as determined for the from standard solution ia not less than 3000 theoretical plates. • Tailing factor for the same peak is not more than 2.0 • The relative standard deviation for peak area • obtained from five replicate injections of standard solution is not more 2.0		
2. Observed Values				
System Precision		Theoretical Plates	5846.44	The column efficiency as determined for the from standard solution is not less than 3000 theoretical plates.
		Tailing Factors	1.30	Tailing factor for the same peak is not more than 2.0
		% RSD	0.65	The % RSD of % assay from Five samples should be more than 2.0
3. **Results :**				
	Sr.No	**Peak Area**	**Theoretical fac**	**Tailing Factor**
	1	2538081	5870.41	1.03

Sr.No	Validation Parameter	Results		Acceptance Criteria
System Suitability and System Precision	2	2525212	5846.21	1.03
	3	2561741	5856.52	1.03
	4	2486453	5849.72	1.03
	5	2496532	5859.23	1.03
	Mean	2532355		
	SD	0.234		
	% RSD	0.03		

Observed Results:

- The observed theoretical plates obtained for the from standard solution is more than 3000 theoretical plates.
- The Observed Tailing factor obtained for the peak from the standard solution is less than 2.0.
- The % RSD of the peak area of obtained from five replica injections of the standard solution is 0.73

Conclusion :
The above data shows that the system is precise.

B. **Method Precision : Method and Procedure**

1. **Methods Precis** Prepared six sample solution of tablets 80 mg as per the test methods and inject into the chromatographic system

Acceptance crit The % RSD of % assay from six samples should be more than 2.0

2. Observed Value

Method Precision	% RSD	0.28	The % RSD of % assay from six samples should be more than 2.0

3. **Results:**

S.No	% Assay
Injection-1	99.5
Injection-2	99.6
Injection-3	99.5
Injection-4	99.5
Injection-5	99.3
Injection-6	98.2
Mean	**99.1**
SD	**0.025**
% RSD	**0.35**

Conclusion :
The above results show that the methods is precise

Accuracy:

The accuracy of an analytical method expresses the closeness of agreement between the value accepted either as a conventional true value or an accepted reference value and the value obtained.

Sr.No	Validation Parameter	Results		Acceptance Criteria
Method and Procedure				
1.	Accuracy was performed by spiking the drugs substance to the placebo at 50%, 100 % and 50% of target concentration of in triplicate at each level and analyzed as per the test method			
	Acceptance Criteria	The % recovery of accuracy levels should be not less than 98.0 and not more than 102		
2. Observed Values				
Accuracy		Mean % recovery	99.87	The % recovery of accuracy levels should be not less than 98.0 and not more than 102.0

3. **Results**

Accuracy level		Accuracy level in mg as	Weight of drug added in mg	% Recovery	Statistical Analysis	
L1 (50 %)	Sample -1	20.54	20.25	100.2	Mean	99.9
	Sample - 2	20.58	20.49	99.9		

Sr.No	Validation Parameter		Results		Acceptance Criteria	
	Sample - 3	20.69	20.55	99.5		
L1 (100 %)	Sample -1	40.44	40.42	100.0	Mean	99.8
	Sample - 2	40.54	40.52	99.8		
	Sample - 3	40.58	40.32	99.8		
L1 (150 %)	Sample -1	60.13	59.64	98.9	Mean	99.9
	Sample - 2	60.24	60.23	99.5		
	Sample - 3	60.155	60.25	100.1		
Overall Statistical Analysis						
Mean		99.9	**SD**	0.30	**% RSD**	0.30
Conclusion : The % recovery of accuracy levels should be not less than 98.0 and not more than 102						

Range

Range of an analytical method is the interval between the upper and lower concentration of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity. The range is normally derived from the linearity studies and depends on the intended application of the procedure.

Sr.No	Validation Param	Results	Acceptance Criter
Method and Procedure			
1.	Range of analytical method can be obtained from linearity, Precision and accuracy data. Report range in % with respect to sample concentration.		
Observed Values			
2.	Range	The analytical method is linear , Precise and accurate from 5 to 150% of target concentration	----
Conclusion : It was concluded from the linearity, Precision and accuracy data that the analytical method is is linear , Precise and accurate from 50% to 150% of target concentration			

Solution Stability

Stability of the analytical solution and extraction time are other parameters which are also evaluated as additional parameters during robustness study. Stability of analytical solution is determined by assessing the results obtained by subjecting the analytical solution to the method parameters for longer period of time e.g. 4 hrs, 12 hrs, 24 hrs, 48 hrs etc.

Sr.No	Validation Parame	Results	Acceptance Criteria
Method and Procedure			
1.	Standard Solution and Sample Solution was prepared as per test methods and stored at refrigerator condition. Solution Stability was evaluated at initial , 12 hours , 24 hours and 48 hours.		
A.	**Acceptance Criteri**	- The overall % RSA from initial replicate standard peaks and bracketing standards peak should be more than 2.0. - The % assay difference from initial and corresponding time intervals should be more than 2	
2.	**Observed Values**		
	Standard Solution	Standard Solution is stable up to hours at refrigerator condition	The overall % RSA from initial replicate standard peaks and bracketing standards peak should be more than 2.0.
	Sample Solution	Sample solution is table up to 48 hours at refrigerator condition	The % assay difference from initial and corresponding time intervals should be more than 2.0
3.	**Results :**		
	Standard Solution :: Over % RSD	Time Interval: Initial	Over all % RSD: 0.18
		12 hours	0.24

Sr.No	Validation Parame	Results		Acceptance Criteria
		24 hours	0.26	
		48 Hours	0.35	
	Standard Solution :: Over % RSD	**Time Interval**	**% Assay**	**Difference of % Assay**
		Initial	98.5	--
		12 hours	99.5	0.4
		24 hours	99.3	0.3
		48 Hours	99.5	1.0

Conclusion : From the above results it is concluded that standard and sample solutions are stable up to 48 Hrs at Refrigerator

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is partially evaluated during method development stages.

Sr.No	Validation Parameter	Results		Acceptance Criteria
Method and Procedure				
1.	**Filter variability**	Prepared three samples solutions as per the test method. One portion of the solution was centrigued and the other portion of sample solution was filtered through two types of filters Nylon and PVDF and calculated the difference of % assay		
	Acceptance Criteria	The difference of % assay compared from centrifuge to the filtered samples should not be more than		
2.	**Observed Values**			
Filter variability		Maximum difference (Centrifuge Vs Nylon)	0.2	The difference of % assay compared from centrifuge to the filtered samples should not be more than 2.0
		Maximum difference (Centrifuge Vs PVDF)	0.2	

3. Results

Sr.No	% Assay			Difference	
	Centrifuge	Nylon	PVDF	Centrifuge Vs Nylon	Centrifuge Vs PVDF
1	99.6	99.7	99.8	0.1	0.2
2	99.7	99.8	99.6	0.1	0.2
3	99.8	99.6	99.6	0.2	0.2

Conclusion :

The Maximum difference Centrifuge Vs Nylon and PVDF membrane filter is 0.4 and 1.6.Hence it is concluded that both Nylon and PVDF filters are suitable for the filtration of the sample solutions.

CONCLUSION

In the current study the effort has been undertaken to improve most simple, economical, sensitive and correct analytical HPLC method for the immediate valuation of these drugs without their prior separation. The method gives resolution with a short analysis time (< 30 min). The method parameter was validated and establish to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the planned method can be used for routine analysis of in medical dosage form.

REFERENCES

1. Kim SH, Kang HC, Lee JS, Kim HD. Rufinamide efficacy and safety in children aged 1-4 years with Lennox-Gastaut syndrome. *Brain Dev.* 2018;40:897–903. [PubMed] [Google Scholar]
2. Saydam M, Takka S. Bioavailability file: rufinamide. *FABAD J Pharm Sci.* 2019;44:231–242. [Google Scholar]
3. Wheless JW, Vazquez B. Rufinamide: a novel broad-spectrum antiepileptic drug. *Epilepsy Curr.* 2010;10:1–6. [PMC free article] [PubMed] [Google Scholar]

4. Cheng-Hakimian A, Anderson GD, Miller JW. Rufinamide: pharmacology, clinical trials, and role in clinical practice. *Int J Clin Pract.* 2006;60:1497–1501. [PubMed] [Google Scholar]
5. Kluger G, Glauser T, Krauss G, Seeruthun R, Perdomo C, Arroyo S. Adjunctive rufinamide in Lennox-Gastaut syndrome: a long-term, open-label extension study. *Acta Neurol Scand.* 2010;122:202–208. [PubMed] [Google Scholar]
6. Wisniewski CS. Rufinamide: a new antiepileptic medication for the treatment of seizures associated with lennox-gastaut syndrome. *Ann Pharmacother.* 2010;44:658–667. [PubMed] [Google Scholar]
7. *The Merck Index. Merck Research Laboratories, 14th edition, (Monograph #3635), 2006; 621.* [Internet]
8. *SC Sweetman (Editor); Martindale. The Complete Drug Reference, 36th edition, The Pharmaceutical Press; London, UK: 2009; 367.* [Internet]
9. McLean AW, Schmutz M, Pozza F. The influence of rufinamide on sodium currents and action potential firing in rodent neurons. *Epilepsia.* 2005;46:296. [Google Scholar]
10. Perucca E, Cloyd J, Critchley D, Fuseau E. Rufinamide: clinical pharmacokinetics and concentration-response relationships in patients with epilepsy. *Epilepsia.* 2008;49:1123–1141. [PubMed] [Google Scholar]
11. Brunner LA, Powell ML. An automated method for the determination of a new potential antiepileptic agent (CGP 33101) in human plasma using high performance liquid chromatography. *Biomed Chromatogr.* 1992;6:278–282. [PubMed] [Google Scholar]
12. Rouan MC, Buffet C, Masson L, Marfil F, Humbert H, Maurer G. Practice of solid-phase extraction and protein precipitation in the 96-well format combined with high-performance liquid chromatography-ultraviolet detection for the analysis of drugs in plasma and brain. *J Chromatogr B Biomed Sci Appl.* 2001;754:45–55. [PubMed] [Google Scholar]
13. la Marca G, Malvagia S, Filippi L, Innocenti M, Rosati A, Falchi M, Pellacani S, Moneti G, Guerrini R. Rapid assay of rufinamide in dried blood spots by a new liquid chromatography-tandem mass spectrometric method. *J Pharm Biomed Anal.* 2011;54:192–197. [PubMed] [Google Scholar]
14. Annapurna MM, Kumar BSP, Goutam SVS, Srinivas L. Stability indicating liquid chromatographic method for the quantitative determination of rufinamide in pharmaceutical dosage forms. *J Drug Deliv Ther.* 2012;2:167–174. [Google Scholar]
15. Annapurna M, Saketha CN, Pavani S. New stability indicating liquid chromatographic method for the quantitative determination of rufinamide in presence of degredant products. *Drug Invent. Today.* 2012;4:501–506. [Google Scholar]
16. Singh J, Sangwan S, Grover P, Mehta L, Kiran D, Goyal A. Analytical method development and validation for assay of rufinamide drug. *J. Pharm. Technol. Res. Managem.* 2013;1:191–203. [Google Scholar]
17. Sai Pavan Kumar B, Mathrusri Annapurna M, Pavani S. Development and validation of a stability indicating RP-HPLC method for the determination of rufinamide. *J Pharm Anal.* 2013;3:66–70.