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Analytical Method Development and Validation Protocol for Antiepileptic Agent – Rufinamide

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Abstract

This study developed and validated a new, sensitive, appropriate, clear, accurate, and robust reversed-phase high-performance liquid chromatography (RP-HPLC) method for determining Rufinamide in bulk medication and tablet formulation. The separation was performed using an HPLC method with a UV detector and Openlab EZ chrome workstation programme, Kromasil C18, 250 mm X 4.6mm ID, 5 µm column, Methanol: 0.025% TFAA (60:40%V/V) was pumped at a flow rate of 1.0 mL/min and detected at 212 nm. The new RP-HPLC method resulted in a 3.22minute retention time for Rufinamide, which was optimized by trial and error. Over a concentration range of 0.5-7.5 µg/mL, the linearity of the established method was confirmed with a correlation coefficient (r2) of 0.99994. The precision of the approach was determined to have a percentage RSD of less than 2.0 percent. The % recoveries were found to be within the acceptable range. The LOD and LOQ were determined to be 0.10 ug/mL and 0.31 ug/mL, respectively. The developed and validated RP-HPLC system takes less time and can be used in the industry for routine quality control/analysis of bulk drug and marketed Rufinamide products.

Keywords: Antiepileptic, Method development, RP-HPLC, Rufinamide, Validation

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Introduction

Rufinamide is an antiepileptic drug approved by the US Food and Drug Administration on November 14, 2008 as an adjunctive treatment of seizures associated with Lennox-Gastaut syndrome in children 4 years and older and adults. Lennox-Gastaut syndrome consists of a variety of treatment-resistant seizures and is most common among pediatric patients.[1-4] Rufinamide, a triazole derivative, was developed in 2004 by Novartis Pharma, AG, and is manufactured by Eisai. It is marketed under the brand name Banzel. It is also marketed in the European Union under the brand name Inovelon.[5,6]

The precise mechanisms by which Rufinamide exerts its antiepileptic effect are unknown. *In vitro* studies suggest that a principal mechanism of action is the modulation of activity in sodium channels, particularly prolongation of the inactive state. In cultured cortical neurons from immature rats, Rufinamide significantly slowed sodium channel recovery from inactivation after a prolonged prepulse and limited the sustained repetitive firing of sodium-dependant action potentials.[7,8]

Chemistry

Rufinamide is chemically known as 1- [(2, 6- difluorophenyl) methyl]-1 H1, 2, 3-triazole-4 carboxamide [Figure 1]. The oral suspension and tablet are bioequivalent on a mg per mg basis. Rufinamide is well absorbed but the rate is slow and the extent of absorption decreases as dose is increases. Based on urinary excretion, the extent of absorption was at least 85% following oral administration of a single dose of 600 mg Rufinamide tablet under fed conditions.[9]

Rufinamide is extensively metabolized but has no active metabolites.[3] Metabolism by carboxyesterases into inactive metabolite CGP 47292, a carboxylic acid derivative, through hydrolysis is the primary biotransformation pathway.[10-15] A few minor additional metabolites were detected in urine, which appeared to be acyl-glucuronides of CGP 47292. The cytochrome P450 enzyme system or glutathiones are not involved with the metabolism of Rufinamide. Rufinamide is a weak inhibitor of CYP 2E1. Rufinamide is a weak inducer of CYP 3A4 enzymes.[11-19]

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Methods

Materials and Reagents

Qualigens (Thermo fisher scientific) provided HPLC grade methanol and trifluoroacetic acid.

Instrumentation and Software

An Agilent 1260 Infinity II HPLC system with DEAX02386 pump and autosampler with UV–visible detector served as the chromatographic system (DEACX16446). For data collection and processing, the chromatograms were registered using Openlab EZ Chrome Workstation on a Windows-based computer system. Rufinamide concentrations were determined using a Kromasil C_{18} column (250 mm × 4.6 mm i.d. 5 µm) column.

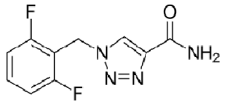

Figure 1: Chemical Structure of Rufinamide

Ultraviolet (UV) Spectroscopy[20-23]

Methanol was selected as the solvent for dissolving Rufinamide. It showed maximum absorbance at 212 nm [Figure 2].

Method Development by RP-HPLC

Selection of analytical wavelength for HPLC method development

Analytical wavelength for the examination was selected from the wavelength of maximum absorption from the spectrophotometric analysis and it was 212 nm. Typical chromatogram of optimized method is shown in Figure 3.

Optimization of HPLC Method

System suitability test (Rufinamide standard solution)

Weighed about 10.2 mg of Rufinamide and transferred in 20 mL volumetric flask, added 15 mL of methanol, sonicate to dissolve it, made volume up to the mark with methanol. Pipette out 0.2 mL from standard stock solution and transferred into 20 mL volumetric flask and made volume up to the mark with mobile phase chromatograms were recorded Typical chromatogram of Standard solution 1 of system suitability solution is shown in Figure 4, Chromatogram of test solution after 24 h is shown in Figure 5, Chromatogram of peak purity of test sample solution is shown in Figure 6 and Results for system suitability test of Rufinamide is shown in Table 1.

Filtration study

This study was conducted with Rufinamide test sample (Physical lab mixture). Filtration study carried out with unfiltered and filtered test solution. During filtration activity 0.45 µm PVDF and 0.45 µm Nylon syringe filters used by discarding 5 mL of aliquot sample.

Stability of analytical solution

Stability study was conducted for standard and test sample solution. Stability study was performed at normal laboratory conditions. The solution was stored at normal illuminated laboratory conditions and analyzed after 12 h and 24 h. Standard and test solution stability study was performed by calculating the difference between results of test solution at each stability time point to that of initial. Optimized Chromatographic Conditions are shown in Table 2.

Results and Discussion

Selection of Analytical Wavelength

Rufinamide STD solution: (20 PPM):

The standard solution was scanned between 400 nm and 200 nm. Wavelength of maximum absorption was determined for drug.

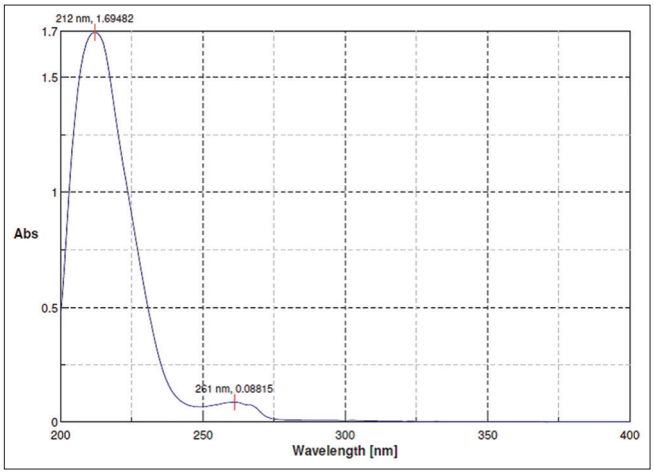

Figure 2: UV spectrum of Rufinamide

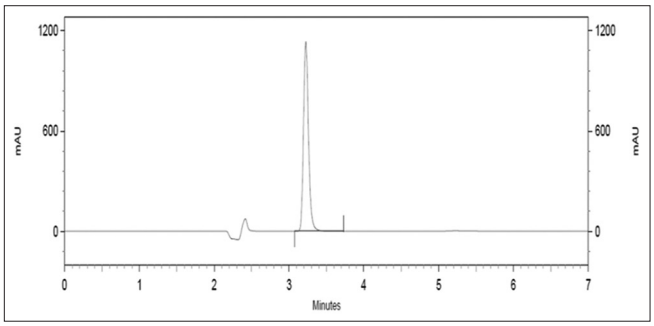

Figure 3: Typical chromatogram of optimized method

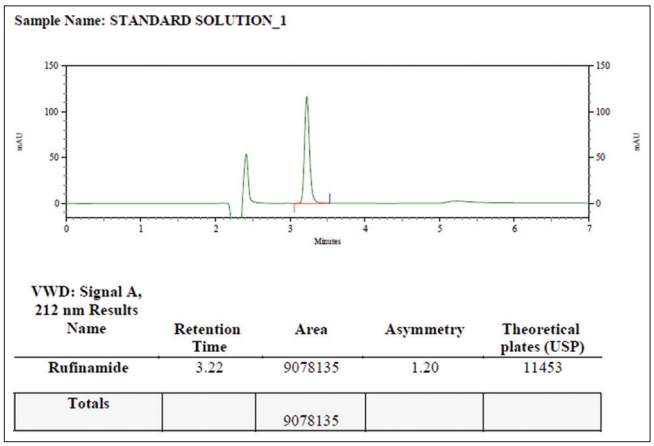

Figure 4: Typical chromatogram of Standard solution 1 of system suitability solution

Rufinamide showed maximum absorbance at 212 nm.

Method Development by RP-HPLC[24-28]

Optimization of HPLC method

System suitability test

From the data tabulated above; the method complies with system suitability parameters.

Filtration study

Filtration study of an analytical procedure checks the interference of extraneous components from filter, deposition on filter bed, and compatibility of filter with sample. Performed on tablet test sample and it is shown in Table 3.

Solution stability

Stability study was conducted for standard as well as test sample. Stability study was performed at normal laboratory conditions. The solution was stored at normal illuminated laboratory conditions and analyzed at initial, after 12 h and 24 h. The results are shown in Table 4.

Table 1: Results for system suitability test of Rufinamide

Parameter	*Acceptance Criteria*	*Result*
%RSD	NMT2.0%.	0.27
Theoretical plates	More than 2000	11450
Tailing factor	NMT2.0	1.2

Table 2: Optimized chromatographic conditions

Parameter	*Description*
Mode	Isocratic
Column Name	Kromasil C18, 250 mm×4.6mm ID, 5 µm
Detector	UV Detector
Injection Volume	20 µl
Wavelength	212 nm
Column Oven Temp	40ºC
Mobile Phase	Methanol: 0.025% TFAA (60:40%V/V)
Flow Rate	1.0 mL/min
Run time	07 min

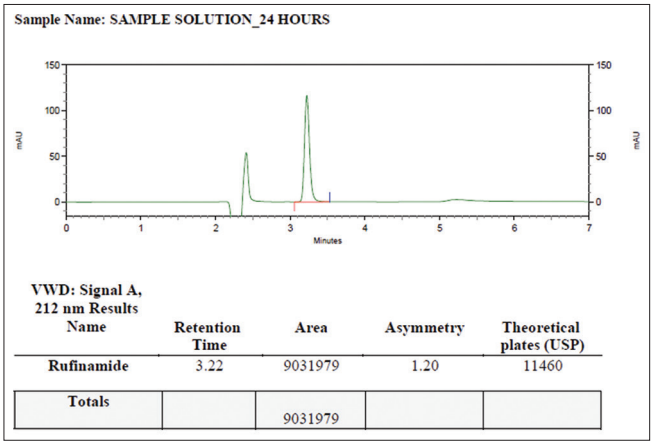

Figure 5: Chromatogram of test solution after 24 h

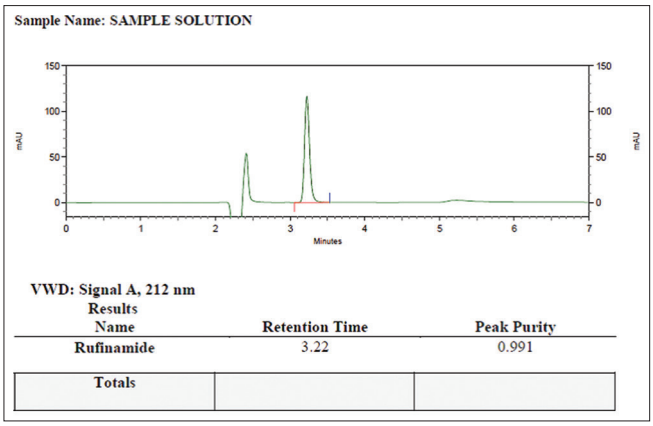

Figure 6: Chromatogram of peak purity of test sample solution

Assay of physical lab mixture

Physical laboratory mixture

Average weight of tablet = 720 mg (Theoretical considered). The assay of physical lab mixture in shown in Table 5.

Validation of RP-HPLC method[24-28]

Specificity

Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present. Blank, standard solution prepared, and injected to check peak purity. The results of specificity is shown in Table 6.

Linearity and range

Linearity of an analytical method is its ability to elicit test results that are proportional to the concentration of analyte in samples within a given range. From the calibration curve, we had to conclude that the Rufinamide shows linear response in the range of 0.5–7.5 µg/mL. The Regression value was found well within the limit Result and statistical data of linearity of Rufinamideis given in Table 7. Linearity data is hown in Table 8, its graph is shown in Figure 7 and Chromatogram of Linearity 150% is shown in Figure 8.

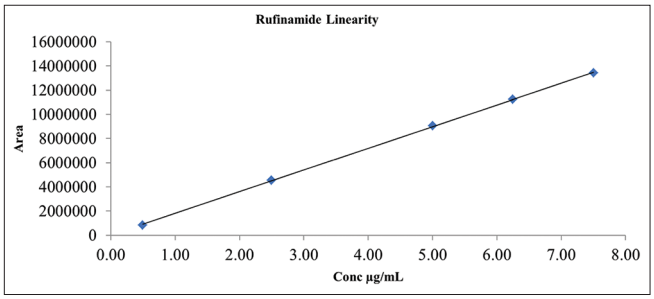

Figure 7: Linearity graph of Rufinamide

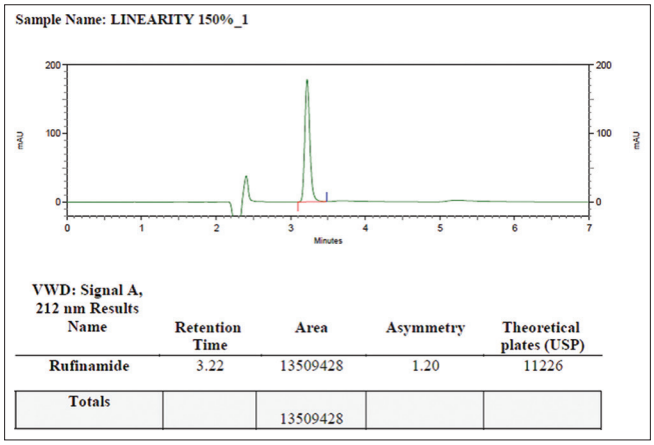

Figure 8: Chromatogram of Linearity 150%

Table 3: Analytical data of filter study of Rufinamide

Sample	*Area*	*%Absolute difference*	*Acceptance Criteria*	*Conclusion*
Unfiltered	9059324	NA	% Absolute difference NMT2.0	Both PVDF and Nylon filters passes the criteria for filter Study
0.45µ PVDF filter	9018425	0.45		
0.45µ Nylon filter	9026721	0.36		

Table 4: Analytical data of Rufinamide for solution stability

Time point	*Test solution*		*Standard solution*		*Acceptance Criteria*	*Conclusion*
	Area	*% Absolute difference*	*Area*	*%Absolute difference*		
Initial	9073014	NA	9082094	NA	%Absolute difference NMT2.0	Both standard and sample solution were found stable for24 h
12 h	9039025	0.37	9053715	0.31		
24 h	9031979	0.45	9043962	0.42		

Table 5: Assay results of physical laboratory mixture

Sample	*Area*	*% Assay*	*Mean Assay*	*Acceptance Criteria*	*Conclusion*
Sample 1	8803675	99.07	99.01	% Assay found should be in the range of 95-105%.	Assay is passed.
Sample 2	8783710	98.96			

Table 6: Results of specificity for Rufinamide

Description	*Observation*	*Acceptance criteria*	*Conclusion*
Blank	No interference at R.T. of Rufinamide due to blank	no interference at R.T.	Developed chromatographic method passed the criteria for specificity.
Placebo	No interference at R.T. of Rufinamide due to placebo	no Interference at R.T.	
Standard solution	Peak purity was 0.996	Peak purity: NLT 0.95	
Test Solution	Peak purity was 0.991	Peak purity: NLT 0.95	

Table 7: Result and statistical data of linearity of Rufinamide

Level	*Conc (µg/mL)*	*Area*	*Mean*	*% RSD*
10%	0.50	853553	893963	0.242
		856301		
		852036		
50%	2.50	4582666	4569775	0.260
		4567358		
		4559301		
100%	5.00	9063682	9040047	0.227
		9030084		
		9026375		
125%	6.25	11285164	11236399	0.392
		11224682		
		11199350		
150%	7.50	13509428	13429822	0.558
		13419243		
		13360794		

Table 8: Data of linearity of Rufinamide

S. No.	*Parameter*	*Result value*	*Acceptance criteria*
1.	Beer's linearity range	0.50–7.50 µg/mL	NA
2.	Correlation coefficient (R^2)	0.99994	NLT 0.98
3.	Intercept	19333.59356	To be report
4.	Slope	1794636.231	To be report
5.	% RSD for area at each level	NA	NMT 2.0

Limit of detection (LOD) and limit of quantitation (LOQ)

Using, σ = 56336.735 (Residual standard deviation of a regression line) and s = 1794636.231 (Slope); LOD is calculated as 0.10 µg/mL and LOQ is calculated as 0.31 µg/mL

Accuracy (recovery)

%Recovery was found well within acceptance range (98.00–102.0%) at all three levels. Result and statistical data of accuracy was given in Table 9. Chromatogram for accuracy is shown in Figure 9.

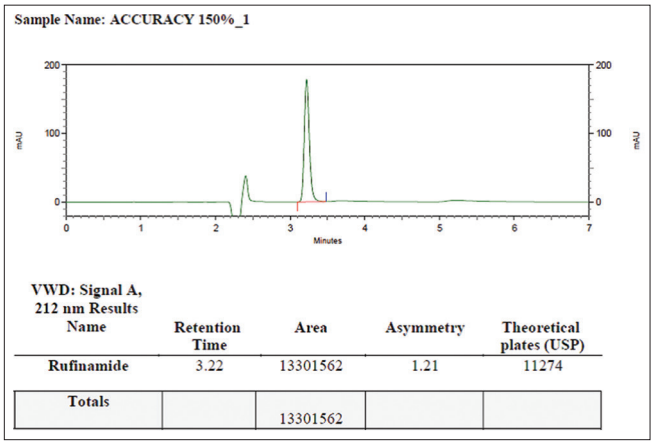

Figure 9: Chromatogram of accuracy

Precision[29]

Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Precision was performed on test sample. Result of intraday and interday precision for Rufinamide test sample assay is shown in Table 10.

Robustness

The robustness of an analytical method 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. Analytical interpretation is given in Table 11.

Table 9: Result and statistical data of accuracy of Rufinamide

Level (%)	*Area*	*% Recovery*	*Mean Recovery*	*% RSD*	*Acceptance Criteria*	*Conclusion*
50	4463075	100.40	99.34	1.0080	% Recovery: 98.00–102.0%	% Recovery was found well within acceptance range at all three levels.
	4425213	99.21				
	4403167	98.41				
100	8803012	98.80	99.13	0.4200		
	8799141	99.00				
	8873018	99.60				
150	13301562	99.73	99.91	0.6788		
	13264829	99.34				
	13420731	100.66				

Table 10: Result of intraday and interday precision for Rufinamide test sample assay

Parameters	*Intraday Precision*	*Interday Precision*	*Acceptance criteria*	*Conclusion*
Mean	99.02	98.95	% RSD for the six samples NMT2.0	HPLC method for the determination of Rufinamide is precise
SD	0.829811	0.609065		
%RSD	0.838	0.616		

Table 11: Analytical data of robustness for Rufinamide

S. No.	*Parameter*	*Observations*						*Limit*
		Changes in flow Rate (mL/min)		*Change in Wavelength (nm)*		*Change in Column Oven temperature (°C)*		
		1.1	*0.9*	*215*	*209*	*42*	*38*	
1.	Theoretical Plate	10393	12560	11413	11405	11412	11391	NMT 2000
2.	Peak area response	8250005	1E+07	8269823	9531264	8829630	8860375	
3.	Tailing factor	1.22	1.18	1.2	1.2	1.23	1.22	NMT 2.0
4.	R.T.(Min)	2.92	3.58	3.22	3.22	3.22	3.22	

Conclusion

The goal of this research was to develop an RP-HPLC system that was simple, dependable, precise, and appropriate. The linearity, accuracy, precision, and robustness of the developed technique of analysis results, as well as the detection and quantification limitations, were all validated. The new method has a number of advantages, including consistent results, quick interpretation, simple sample preparation, and better selectivity and sensitivity. Because it is reliable, reproducible, and time-saving, the developed approach can be employed for routine research in the pharmaceutical sector for the bulk medication Rufinamide as well as pharmaceutical dose types. According to the above experimental results, this newly developed method for estimating Rufinamide was found to be simple, precise, and accurate, with a shorter retention time, making it more acceptable and cost-effective for routine analysis in research institutions, quality control departments in industries, and approved testing laboratories.

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