Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(10):306-314

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Development and Validation of Related Substances Method for Rufinamide Tablets by RP-HPLC

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ABSTRACT

The present paper describes about highly specific, linear, precise, rugged, accurate, robust and stability indicating RP-HPLC method for determination of related substances present in rufinamide tablets. Chromatographic separation with separation of impurities at satisfactory level was achieved using Inertsil ODS-3V (250 × 4.6 mm, 5 µm) column by using gradient elution mode. Mobile phase-A consists of 0.1% v/v triethylamine in water. pH adjusted to 2.2 with orthophosphoric acid. Mixture of 980 ml of methanol and 20 ml of tetrahydrofuran was used as Mobile phase-B. Flow rate was kept at 1.0 ml/min with a monitoring wavelength of 215 nm. Developed method was successfully validated as per method validation parameters recommended by International Conference on Harmonisation (ICH) for specificity, LOQ, LOD, linearity, precision, accuracy, robustness and solution stability. The validated Reverse phase-High Performance Liquid Chromatography (RP-HPLC) method was successfully used for quantitative determination of related substances of present and stability batches of rufinamide tablets.

Keywords: Rufinamide; RP-HPLC; Validation

INTRODUCTION

Rufinamide [1] (Figure 1) is a triazole derivative and an anticonvulsant medication to treat seizure disorders like Lennox-Gastuat syndrome, a form of childhood epilepsy. It works by modulation of the activity of sodium channels and in particular, prolongation of the inactive state of the voltage gated sodium channels thus stabilising membranes, ultimately blocking the spread of partial seizure activity. It works by decreasing abnormal excitement in the brain [2,3].

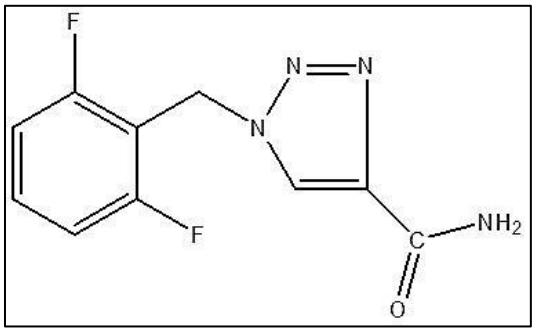

Figure 1: Structure of rufinamide (1-(2,6-difluorobenzyl)-1H-1,2,3-triazole-4-carboxamide)

Two RP-HPLC methods [4,5] were reported for determination of impurities in rufinamide API. No methods are available for determination of impurities present in rufinamide tablets. Impurities present in drug product depend on synthetic route of active pharmaceutical ingredient and different processes followed for manufacturing. So existing methods are rarely useful for determination of impurities. Since HPLC is most available instrument in quality control laboratories with better selectivity and sensitivity it was proposed to develop gradient method based on RP-HPLC mode. In the present study authors report a specific, linear, precise, rugged, accurate and robust RP-HPLC method for determination of impurities present in present and stability batches of rufinamide tablets.

MATERIALS AND METHODS

Chemicals and Solvents

Methanol HPLC grade and Triethylamine HPLC grade were procured from E.Merck (India) Ltd., Mumbai, India. Orthophosphoric acid HPLC grade was obtained from Qualigens Fine Chemicals Pvt. Ltd, India. Tetrahydrofuran HPLC grade was obtained from Himedia Laboratories, India. Hydrochloric acid, Sodium hydroxide and Hydrogen Peroxide HPLC grade were obtained from Loba Chemie Pvt. Ltd, India. Water was obtained from Milli Q water purification system.

Working Standards and Sample

Rufinamide Working Reference Standard (WRS) Potency-99.7%, Impurity-A (1-(2-fluorobenzyl)-1H-1,2,3-triazole-4-carboxamide) WRS Potency-98.9% , Impurity-B (Methyl 1-(2,6-difluoro benzyl)-1H-1,2,3-triazole-4-carboxylate) WRS Potency-99.6%, Impurity-C (1-(2,6-difluorobenzyl)-1H-1,2,3-triazole-4-carboxilic acid) WRS Potency-99.5% and rufinamide tablets 200 mg were provided by Ricon Pharma India Private Limited, Hyderabad as gift samples.

Preparation of Mobile Phase-A

1 ml of triethylamine was added to 1000 ml of water, mixed well and pH was adjusted to 2.2 with orthophosphoric acid.

Mobile Phase-B

Methanol and tetrahydrofuran were mixed well in 98:2% v/v.
Diluent: Water and methanol were mixed in 50:50% v/v.

Chromatographic Conditions

Related substances method development [6-8] and validation were carried out on a Waters Alliance 2695 Seperations module with 2996 PDA detector, a 100 µL injection loop, auto sampler and running on Empower-2 software. The chromatographic conditions are as follows,
Column: Inertsil ODS-3V (250 × 4.6 mm), 5 µ
Flow rate: 1.0 ml/min
Detection wavelength: 215 nm
Injection volume: 50 µl
Runtime: 90 minutes
Column temperature: 35°C
Gradient programme is shown in Table 1.

Table 1: Gradient programming

Time in minutes	% of Mobile phase-A	% of Mobile phase-B
0.01	90	10
12	65	35
25	65	35
60	45	55
80	45	55
82	90	10
90	90	10

Preparation of Standard Stock Solution
50.05 mg of rufinamide working reference standard was weighed and transferred into a 100 ml volumetric flask. To this 50 ml of the diluent was added and sonicated for 5 minutes and volume was made up to the mark with the diluent. 5 ml of the above solution was pipette into a 100 ml volumetric flask and volume was made up to the mark with the diluent.

Preparation of Standard Solution
1 ml of standard stock solution was pipetted into a 100 ml volumetric flask and diluted to the mark with the diluent.

Preparation of Impurity A Stock Solution
5.02 mg of impurity A WRS was weighed and transferred into a 100 ml volumetric flask. 50 ml of the diluent was added and sonicated for 5 minutes and diluted to the volume with the diluent.

Preparation of Impurity A Solution
5 ml of impurity A stock solution was pipetted into a 50 ml volumetric flask and diluted to the volume with the diluent.

Preparation of Impurity B Stock Solution
5.04 mg of impurity B WRS was weighed and transferred into a 100 ml volumetric flask. 50 ml of the diluent was added and sonicated for 5 minutes and diluted to the volume with the diluent.

Preparation of Impurity B Solution
5 ml of impurity B stock solution was pipetted into a 50 ml volumetric flask and diluted to the volume with the diluent.

Preparation of Impurity C Stock Solution
5.01 mg of impurity C WRS was weighed and transferred into a 100 ml volumetric flask. 50 ml of the diluent was added and sonicated for 5 minutes and diluted to the volume with the diluent.

Preparation of Impurity C Solution
5 ml of impurity C stock solution was pipetted into a 50 ml volumetric flask and diluted to the volume with the diluent.

Preparation of Reference Solution
50.05 mg of rufinamide working reference standard was accurately weighed and transferred into a 100 ml volumetric flask. 50 ml of the diluent was added and sonicated for 5 minutes. To the above solution 0.5 ml each of impurity stock A, impurity stock B and impurity stock C were added and diluted to the volume with the diluent.

System Suitability
Reference solution and standard solutions were used to evaluate system suitability.
Acceptance criteria

- USP plate count (N) for each impurity and main peak from reference solution should be not less than 5000.
- USP tailing factor (T) for each impurity and main peak from reference solution should be not more than 2.0.
- USP resolution between rufinamide and impurity-A from reference solution should be more than 1.5.
- The % RSD of six peak areas from replicate standard injections should be not more than 5.0.

Preparation of Sample Solution
20 tablets were weighed and average weight of tablet was determined. Tablets were crushed into a fine powder. 136.75 mg of tablet powder equivalent to 50 mg of rufinamide was weighed accurately and transferred into a 100 ml volumetric flask. 50 ml of the diluent was added and sonicated for 20 minutes with intermittent shaking. Volume was made up to the mark with the diluent and centrifuged at 5000 RPM for 10 minutes. Clear supernatant solution was used as sample solution.

Method Validation
The proposed method for related substances was subjected to validation [9,10] as per ICH guidelines to test its suitability for intended purpose.

Specificity
Specificity was demonstrated by comparing blank, reference, standard, individual impurities, sample and spiked sample chromatograms. Chromatogram for system suitability and results for system suitability were shown in Figures 2 and 3 and Table 2. Forced degradation study was performed by stressing sample with acid, base, peroxide, heat, light and humidity. Base degradation chromatogram and results were shown in Figure 4 and Table 3.

Limit of Quantitation (LOQ) and Limit of Detection (LOD)
A series of standard solutions containing rufinamide, impurity-A, impurity B, impurity-C below 50% of the specification limit were prepared and analysed. LOQ and LOD were established based on from the residual standard deviation on response 'Se' (standard error of the predicted y-value for each x in a regression) and slope. LOQ chromatogram and results were shown in Figure 5 and Table 4. LOD Chromatogram and results were shown in Figure 6 and Table 5.

Linearity
Linearity was demonstrated by plotting curves from LOQ to 150% of the impurity specification level. Slope, Intercept and correlation coefficient were calculated for each impurity and main analyte. Linearity graphs for impurities and main analyte plotted between concentrations vs. peak area were shown in Figures 7-10. Results for linearity were shown in Table 6.

Precision
System precision was demonstrated by injecting six replicate injections of standard solution. Method precision was demonstrated by injecting a set of six separate samples spiked with impurities at specification level. Ruggedness for method was demonstrated by injecting a set of six separate samples at specification level into different chromatographic system by different analyst on different day using different column. Percentage relative standard deviations (%RSD) for peak areas were calculated for system precision. % RSD for Percentage weight by weight of impurities was calculated for method precision and intermediate precision. Chromatogram of method precision for spiked sample was shown in Figure 11 and results for system precision, method precision and intermediate precision were shown in Table 7.

Accuracy
Accuracy was performed in triplicate by spiking impurities into sample at 50%, 100% and 150% of specification level. % mean recoveries at each level for all impurities were shown in Table 8.

Range
Range of the method was proposed using data from linearity and accuracy.

Robustness
Robustness was evaluated from reference solution by changing flow rate (± 10% of actual flow rate), organic content (± 2% absolute in organic content of mobile phase) and column temperature (± 5°C).

Solution Stability
Solution stability for reference solution and sample solution spiked with impurities was evaluated at room temperature (25°C).

RESULTS AND DISCUSSION

System Suitability Evaluation

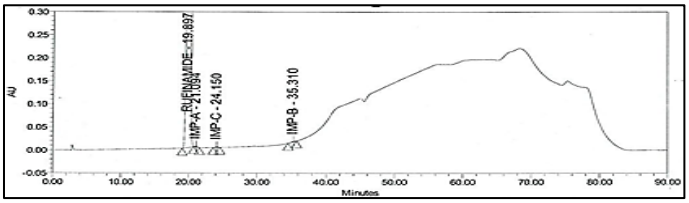

Figure 2: Reference chromatogram

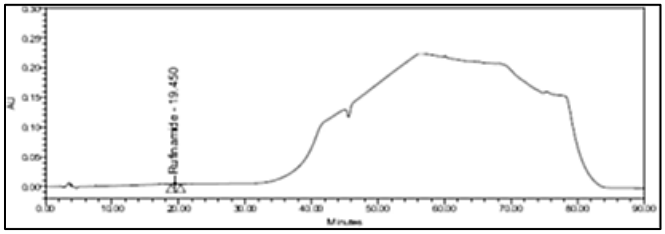

Figure 3: Standard chromatogram

Table 2: System suitability results

From Reference Solution					
S.No	**Peak Name**	**Retention time (min)**	**USP Plate Count**	**USP Tailing**	**USP Resolution**
1	Rufinamide	19.897	47025	1.05	-
2	Impurity-A	21.094	14590	1.22	1.71
3	Impurity-C	24.15	16150	1.14	4.46
4	Impurity-B	35.31	15495	0.91	13.81
From Standard Solution					
% RSD of six standard peak areas-0.2%					

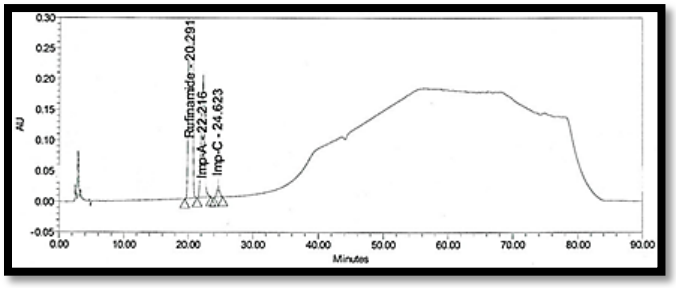

Figure 4: Base degradation chromatogram

Table 3: Base degradation results

S. No	Name	Retention time (Minutes)	Area	% Area	Purity angle	Purity threshold
1	Rufinamide	20.291	115201219	89.85	4.648	11.916
2	Impurity-A	22.216	11949642	9.32	0.189	0.375
3	Impurity-C	24.623	1064185	0.83	0.312	0.418
Total Area			128215046			

Limit of Quantitation

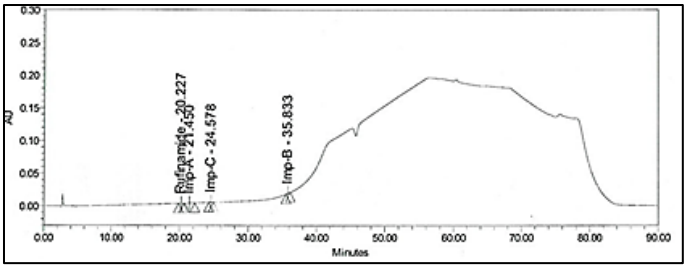

Figure 5: Chromatogram of LOQ

Table 4: Results of LOQ

Name of the peak	Retention time (min)	LOQ Concentration (µg/ml)
Rufinamide	20.227	0.014
Impurity-A	21.45	0.0378
Impurity-C	24.578	0.0217
Impurity-B	35.833	0.0165

Limit of Detection

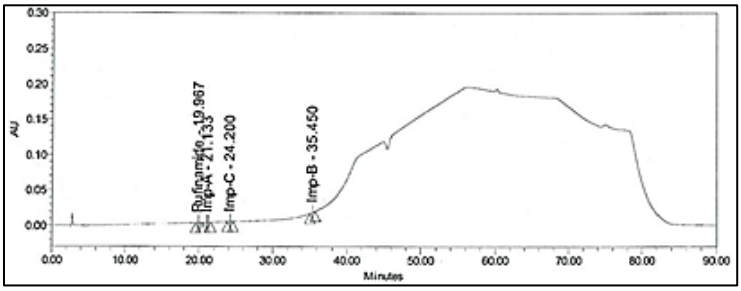

Figure 6: Chromatogram of LOD

Table 5: Results of LOD

Name of the peak	Retention time (min)	LOD Concentration (µg/ml)
Rufinamide	19.967	0.0046
Impurity-A	21.133	0.0125
Impurity-C	24.2	0.0072
Impurity-B	35.45	0.0055

Linearity

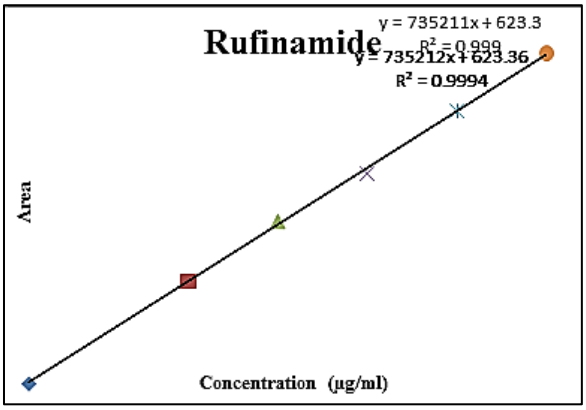

Figure 7: Linearity graph of rufinamide

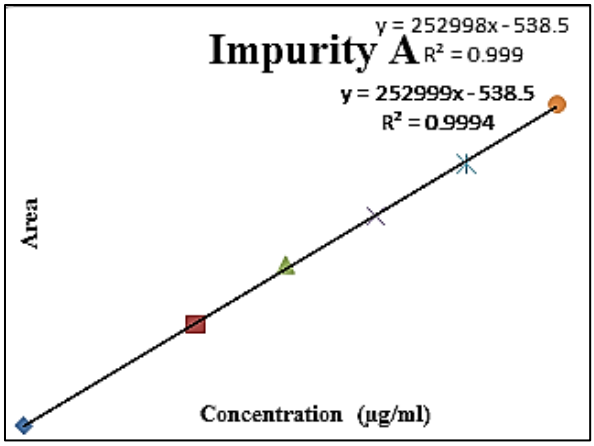

Figure 8: Linearity graph of impurity-A

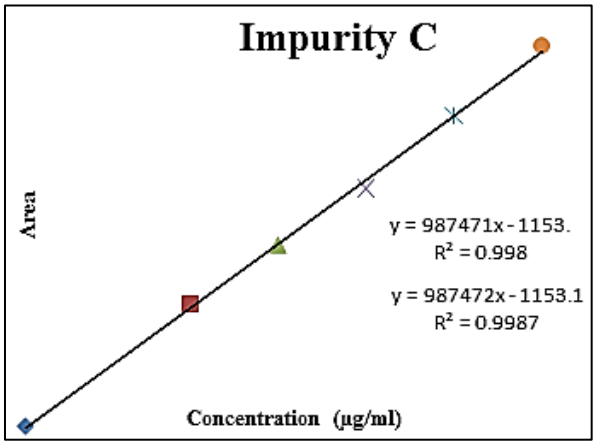

Figure 9: Linearity graph of impurity-C

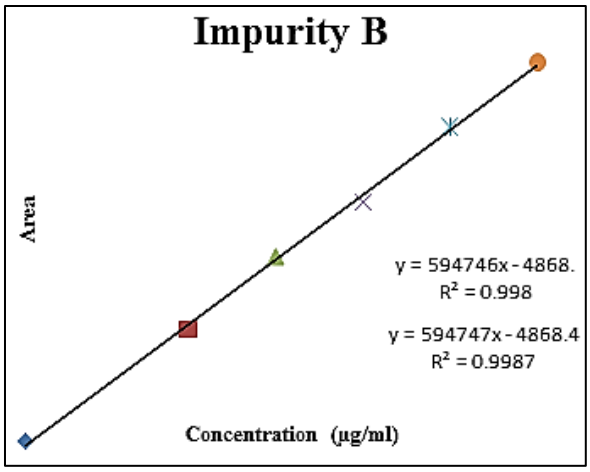

Figure 10: Linearity graph of impurity-B

Table 6: Results of linearity study

Name	Slope	Intercept	Correlation coefficient
Rufinamide	735211.82	623.35789	0.9997
Impurity-A	252998.98	-538.50094	0.9997
Impurity-C	987471.94	-1153.0918	0.9993
Impurity-B	594746.8	-4868.407	0.9994

Precision

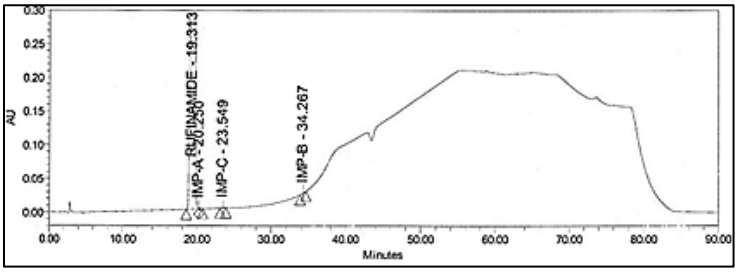

Figure 11: Chromatogram of spiked sample of method precision

Table 7: Results of system precision, method precision and intermediate precision

Name	System Precision* (%RSD) *	Method Precision** (%RSD) **	Intermediate Precision*** (%RSD) ***
Rufinamide	0.2	-	-
Impurity-A	-	2.9	2.8
Impurity-C	-	0	0
Impurity-B	-	0	0

*%RSD of peak areas from six replicat injections

**%RSD of % w/w of each impurity from six samples

***%RSD of % w/w of each impurity from twelve samples

Accuracy

Table 8: Results for accuracy study

Name	%Mean Recovery			Range
	50%	**100%**	**150%**	50%
Impurity-A	99.1	99.6	99.9	to 150% of the specification level
Impurity-C	94.6	98	98.5	
Impurity-B	95.9	97.9	99	

Forced degradation studies were performed to prove that the method is stability indicating. In base degradation, impurity-C was observed and all peaks were found to be pure. The LOD and LOQ values were calculated for both main analyte and impurities. The calculated LOQ values allow confidant determination of impurities by the proposed RP-HPLC method. Linearity results revealed that correlation coefficients for main peak and all impurities were more than 0.990 indicates linearity of the proposed method. In system precision study the %RSD of main peak areas was found to be below 5. The %RSD of impurities in method precision and intermediate precision (ruggedness) studies were found to be below 10 indicates that the proposed method is precise. The % mean recovery values for all impurities at 50%, 100% and 150% of specification levels were found to be 94.6-99.9%. It indicates high recovery of impurities from their matrix. Range was demonstrated from 50% to 150% of the specification level. System suitability was passed against variable changes in flow rate, organic content and column temperature. This indicates high robustness of the proposed method. The reference solution and spiked sample solution were stable for 48 hours at room temperature (25°C).

CONCLUSION

The developed method was found to be highly specific, linear, precise, rugged, accurate, robust and stability indicating for determination of related substances of rufinamide tablets and can be reliably adopted for quality control analysis of present and stability batches of rufinamide tablets.

ACKNOWLEDGEMENTS

The author wish to thank the management of Ricon Pharma India Private Limited, Hyderabad, India for supporting this work.

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