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RESEARCH ARTICLE

STABILITY INDICATING LIQUID CHROMATOGRAPHIC METHOD FOR THE QUANTITATIVE DETERMINATION OF RUFINAMIDE IN PHARMACEUTICAL DOSAGE FORMS

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Received 13 June 2012; Review Completed 14 July 2012; Accepted 14 July 2012, Available online 15 July 2012

ABSTRACT

A selective, specific and sensitive stability-indicating high-performance liquid chromatographic method was developed and validated for the determination of Rufinamide in tablet dosage forms. Reversed-phase chromatography was performed on Shimadzu Model CBM-20A/20 Alite, equipped with SPD M20A prominence photodiode array detector using C18 column (250 mm × 4.6 mm, 5 µm) with a flow rate of 1.0 mL/min. Detection wavelength was 210 nm and temperature was 30°C. Linearity was observed in the concentration range of 0.01–160 µg/mL with regression equation y = 112887 x + 35285 with correlation coefficient of 0.9998. The LOQ and LOD were found to be 0.0086 µg/mL and 0.0028 µg/mL respectively. The percentage relative standard deviation in precision and accuracy studies was found to be less than 2%. Rufinamide was subjected to stress conditions of degradation in aqueous solutions including acidic, alkaline, oxidation, photolysis and thermal degradation and it was found that the drug is highly resistant towards all degradations as the decomposition was less than 1.5%. The developed method was validated with regard to linearity, accuracy, precision, selectivity and robustness and the method was found to be precise, accurate, linear and specific. As the proposed LC method achieved satisfactory resolution between Rufinamide, its degradation products, intermediate product possibly present in Rufinamide drug substance and other impurities in the end product before refining in the final step of synthetic process, it can be employed as a stability indicating one, used for the synthetic process control and determination of Rufinamide in pharmaceutical preparations.

Keywords: Rufinamide, Isocratic elution, RP-HPLC, Validation, Stability-indicating, LOD, LOQ.

INTRODUCTION

Rufinamide is chemically known as 1- [(2, 6-difluorophenyl) methyl]-1H-1,2,3-triazole-4 carboxamide with molecular formula $C_{10}H_8F_2N_4O$ and molecular weight 238.19 g/mol as shown in Figure 1. Rufinamide is an antiepileptic drug approved by the US Food and Drug Administration as 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 paediatric patients [1]. The mechanism of action of Rufinamide is unknown but it is presumed to involve stabilization of the sodium channel inactive state, effectively keeping the ion channels closed. It is believed to prolong the refractory period of voltage-dependent sodium channels, making neurons less likely to fire [2].

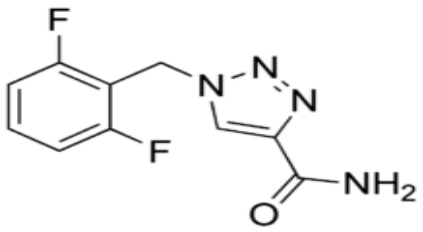

Figure 1: Chemical Structure of Rufinamide

Very few methods are reported in the literature regarding the clinical studies and no stability indicating method is available in the official compendia using HPLC for analysing Rufinamide in dosage forms. Analytical methods for Rufinamide from pharmaceutical dosage form should be developed and validated. To date, all analytical methods described in literature for the determination of Rufinamide in biological fluids involve liquid chromatography 3-7 and liquid chromatography–mass spectrometry methods 8-9.

Quality control of pharmaceutical products requires identification and quantification of the active ingredient and its impurities for safety and efficacy reasons. Impurities and potential degradation products that may exist in medicines can change the chemical, pharmacological and toxicological properties of the product. Since pharmacopoeias do not describe a suitable method for the determination of Rufinamide in pharmaceutical formulations, in the present work we developed simple, rapid and accurate reverse phase liquid chromatographic method for the determination of Rufinamide tablets as an alternative method. Apart from this, it can be used for assays of Rufinamide in biological fluids or in pharmacokinetic investigations.

MATERIALS AND METHODS

Chemicals and Reagents

Rufinamide standard (purity ≥ 98.0%) was obtained from Eisai Pharmaceuticals (Visakhapatnam, India). Methanol

(HPLC grade), sodium hydroxide (NaOH) and hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2) were purchased from Merck (India).

Rufinamide is available as tablets with brand names ^{Pr}BANZEL™® as tablets (Label claim: 200 and 400 mg) and BANZEL® as tablets (Label claim: 200 and 400 mg) as well as oral suspension (Label claim: 40 mg/mL) . All chemicals were of analytical grade and used as received.

Instrumentation

Chromatographic separation was achieved by using a Shimadzu Model CBM-20A/20 Alite HPLC system, equipped with SPD M20A prominence photodiode array detector (250 mm × 4.6 mm, 5 µm particle size) maintained at 25 °C.

Chromatographic Conditions

Isocratic elution was performed using methanol and water (52:48, V/V) with flow rate 1.0 mL/min. 20 µL of sample was injected into the HPLC system.

Preparation of Rufinamide Stock Solution

Rufinamide stock solution (1000 µg/mL) was prepared by accurately weighing 25 mg of Rufinamide in a 25 mL amber volumetric flask and making up to volume with mobile phase. Working solutions for HPLC injections were prepared on a daily basis from the stock solution in a solvent mixture of methanol and water (52:48, V/V) (mobile phase). Solutions were filtered through a 0.45 µm membrane filter prior to injection.

Preparation of Sample Solutions

Twenty tablets from each brand (^{Pr}BANZEL™® and BANZEL®) were procured, weighed and crushed to a fine powder. Powder equivalent to 25 mg Rufinamide was accurately weighed into a 25 ml volumetric flask and made up to volume with mobile phase. The contents of the volumetric flask were sonicated for 30 min to enable complete dissolution of Rufinamide. The solution was filtered and the filtrate was diluted with mobile phase. 20 µL of these solutions were injected into the system and the peak area was recorded from the respective chromatogram.

Forced Degradation Studies/Specificity

The study was intended to ensure the effective separation of Rufinamide and its degradation peaks of formulation ingredients at the retention time of Rufinamide. Separate portions of drug product and ingredients were exposed to the following stress conditions to induce degradation. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method [10]. All solutions for use in stress studies were prepared at an initial concentration of 1 mg/mL of Rufinamide and refluxed for 30 min at 80 °C. All samples were then diluted in mobile phase to give a final concentration of 80 µg/mL and filtered before injection.

Acidic and Alkaline Degradation

Acid decomposition was carried out in 0.1 M HCl at a concentration of 1.0 mg/mL Rufinamide and after refluxation for 30 min at 80 °C the stressed sample was cooled, neutralized and diluted with mobile phase to give a final concentration of 80 µg/mL and filtered before injection.

Similarly stress studies in alkaline conditions were conducted using a concentration of 1.0 mg/mL in 0.1 M NaOH and refluxed for 30 min at 80 °C. After cooling the solution was neutralized and diluted with mobile phase to give a final concentration of 80 µg/mL and filtered before injection.

Oxidative Degradation

Solutions for oxidative stress studies were prepared using 3% H_2O_2 at a concentration of 1 mg/mL of Rufinamide and after refluxation for 30 min at 80 °C on the thermostat the sample solution was cooled and diluted with the mobile phase to give a final concentration of 80 µg/mL and filtered before injection.

Thermal Degradation

For thermal stress testing, the drug solution (1 mg/mL) was heated in thermostat at 80 ºC for 30 min, cooled and diluted with the mobile phase to give a final concentration of 80 µg/mL and filtered before injection.

Photolytic Degradation

The drug solution (1 mg/mL) for photo stability testing was exposed to UV light for 4 hours UV light chamber (365 nm) and diluted with the mobile phase to give a final concentration of 80 µg/mL and filtered before injection.

Method Validation

The method was validated for the following parameters: system suitability, linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity and robustness [11].

Linearity

Linearity test solutions for the assay method were prepared from a stock solution at different concentration levels of the analyte (0.01-160 µg/mL). 20 µL of each solution was injected in to the HPLC system and the peak area of the chromatogram obtained was noted.

The solutions extracted from the marketed formulations were injected in to the HPLC system and the peak area of the chromatograms was noted. The analytical curve was evaluated on three different days. The peak area vs. concentration data was analyzed with least squares linear regression. The slope and y-intercept of the calibration curve was reported.

Precision

The intra-day precision of the assay method was evaluated by carrying out 9 independent assays of a test sample of Rufinamide at three concentration levels (20, 40 and 80 µg/mL) (n=3) against a qualified reference standard. The %RSD of three obtained assay values at three different concentration levels was calculated. The interday precision study was performed on three different days i.e. day 1, day 2 and day 3 at three different concentration levels (20, 40 and 80 µg/mL) and each value is the average of three determinations (n=3). The % RSD of three obtained assay values on three different days was calculated.

 ISSN: 2250-1177 CODEN (USA): JDDTAO

Accuracy

The accuracy of the assay method was evaluated in triplicate at three concentration levels (80, 100 and 120%), and the percentage recoveries were calculated. Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of Rufinamide in the drug product. The study was carried out in triplicate at 18, 20 and 22 µg/mL. The percentage recovery in each case was calculated.

Sensitivity/Limit of quantification (LOQ) and limit of detection (LOD)

The limit of quantification (LOQ) and limit of detection (LOD) were based on the standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in International Conference on Harmonization guidelines Q2 (R1) [11]. Sensitivity of the method was established with respect to limit of detection (LOD) and LOQ for Rufinamide. LOD and LOQ were established by slope method as mentioned below.

$$LOD = \frac{3.3 \times \text{standard deviation of y-intercept}}{\text{Slope of the calibration curve}}$$

$$LOQ = \frac{10 \times \text{standard deviation of y-intercept}}{\text{Slope of the calibration curve}}$$

LOD and LOQ were experimentally verified by injecting six replicate injections of each impurity at the concentration obtained from the above formula.

Robustness

The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (208 and 212 nm), percentage of acetonitirile in the mobile phase (54 and 50) and flow rate (0.9 and 1.1 mL/min). Robustness of the method was studied using six replicates at a concentration level of 20 µg/mL of Rufinamide.

Solution Stability and Mobile Phase Stability

The solution stability of Rufinamide in the assay method was carried out by leaving both the sample and reference standard solutions in tightly capped volumetric flasks at room temperature for 48 h. The same sample solutions were assayed at 12 h intervals over the study period. The mobile phase stability was also assessed by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions at 12 h intervals up to 48 h. The prepared mobile phase remained constant during the study period. The % RSD of the Rufinamide assay was calculated for the mobile phase and solution stability experiments. An additional study was carried out using the stock solution by storing it in a tightly capped volumetric flask at 4 °C.

RESULTS AND DISCUSSION

No stability indicating method is available in the official compendia using HPLC for analysing Rufinamide in dosage forms till now. The present proposed method is simple, precise and accurate in comparison to the reported methods in the literature (Table 1).

A reversed-phase chromatographic technique was developed to quantitate Rufinamide at 210 nm. Methanol was chosen as an organic modifier in the mobile phase. Satisfactory resolution was achieved with use of a mixture of methanol and water (52:48, V/V) as demonstrated in Figure 2. C8 and C18 columns were first evaluated as stationary phase for the separation of Rufinamide. C18 column was adopted for the analysis because it provided a better separation of the analytes.

Table 1 Comparison of the performance characteristics of the present method with the published methods

S. No.	Method /Reagent	λ (nm)	Linearity (µg/mL)	Remarks	Ref.
1.	HPLC (Robotic system)	230	0.05–4.0	Human Plasma	3
2.	HPLC Acetonitrile: methanol: potassium dihydrogen phosphate	-	0.05-19.09	Plasma (Liquid-solid extraction)	4
3	HPLC Acetonitrile: methanol : Potassium dihydrogen phosphate	-	0.05–20	Plasma and brain	5
4	HPLC Acetonitrile: methanol Potassium dihydrogen phosphate buffer (pH 4.5)	210	2-40	Very narrow linearity range (UV/visible detector)	6
5	HPLC Methanol: dichloromethane:n-hexane	230	0.25–20.0	Plasma and Saliva	7
6	LC-MS	-	0.48–47.6	dried blood spots	8
7	HPLC Methanol: water (Adjusted to pH 3.0 with ortho phosphoric acid)	220	10-60	Very narrow linearity range	9
8	HPLC Methanol: water (52:48, V/V)	210	0.01-160	Stability indicating method Wide linearity range (PDA detector)	Present work

 ISSN: 2250-1177 CODEN (USA): JDDTAO

The methods reported in the literature are applicable only for bioanalytical determination of Rufinamide. The present method is a stability indicating RP-HPLC method which was not reported earlier.

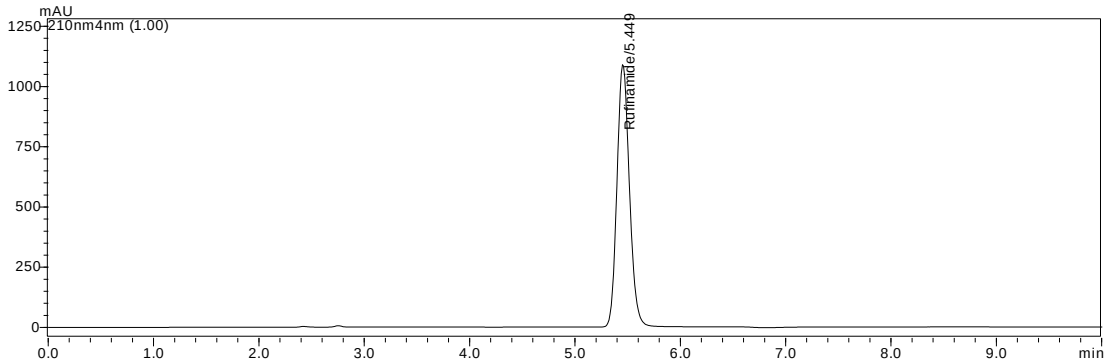

Figure 2: Typical Chromatogram of Rufinamide (80 µg/mL)

The present stability-indicating method for the determination of Rufinamide in pharmaceutical formulations is specific because the drug peak was well separated even in the presence of degradation products. Overall, the data demonstrated that the excipients and the degradation products did not interfere with the Rufinamide peak, indicating the selectivity of the method. The complete separation of the analytes was accomplished in less than 10 min and the method can be successfully applicable to perform long-term and accelerate stability studies of Rufinamide formulations.

HPLC Method Development and Optimization

Initially the stressed samples were analyzed using a mobile phase consisting of water: acetonitrile (70:30, v/v) at a flow rate of 1.0 mL/min. Under these conditions, the resolution and peak symmetry were not satisfactory, so the mobile phase was changed to methanol: water (52:48, V/V) with a flow rate of 1.0 mL/min under which peaks were well resolved with good symmetry and sharpness. Therefore, mobile phase containing methanol: water (52:48, V/V) was chosen for the best chromatographic response for the entire study.

Method Validation

System Suitability

The system suitability test was performed to ensure that the complete testing system was suitable for the intended application. The parameters measured were peak area, retention time, tailing factor, capacity factor and theoretical plates. In all measurements the peak area varied less than 2.0%, the average retention time was 5.5 ± 0.05 minutes. The capacity factor was more than 2, theoretical plates were 9873 (more than 2000) and tailing factor was 1.22 (less than 2) for the Rufinamide peak. The proposed method offers high sensitivity and Rufinamide can be detected accurately. In all the cases, the Rufinamide peak was well separated from the degradation products.

Linearity

The calibration curve for Rufinamide was linear over the concentration range of 0.01–160 µg/mL. The data for the peak area of the drug in corresponds to the concentration was treated by linear regression analysis (Table 2) and the regression equation for the calibration curve (Figure 3) was found to be y = 112887 x + 35285 with correlation coefficient of 0.9998.

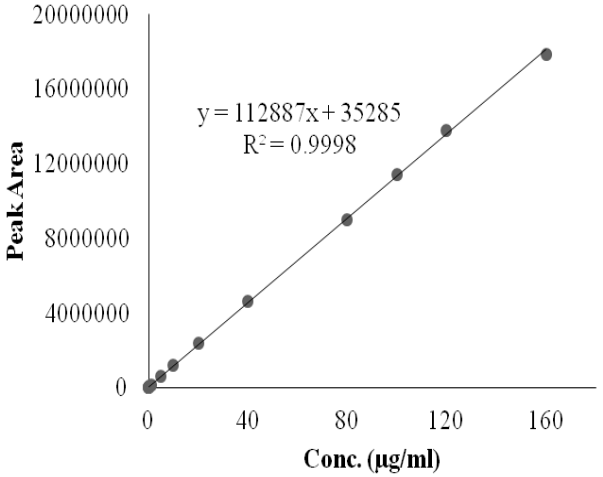

Figure 3: Calibration Curve of Rufinamide

Table 2: Linearity of Rufinamide

Conc. (µg/mL)	*Mean area ± SD	RSD (%)
0.01	1490.33±5.033	0.34
0.05	5816.33±15.18	0.26
0.1	12298.33±25.17	0.21
0.5	59028.00±136.57	0.23
1	120509.00±552.39	0.46
5	584986.00±1660.33	0.28
10	1183090.33±4199.83	0.35
20	2362078.66±4904.92	0.21
40	4554834.66±10664.88	0.23
80	9026285.00±11646.05	0.29
100	11437163.66±58773.76	0.51
120	13771048.00±77113.34	0.56
160	17849924.00±123972.63	0.69

Precision

The precision of the method was determined by repeatability (Intra-day precision) and intermediate precision (Inter-day precision) of the Rufinamide standard solutions. Repeatability was calculated by assaying three samples of each at three different concentration levels (20, 40 and 80 µg/mL) on the same day. The inter-day

precision was calculated by assaying three samples of each at three different concentration levels (20, 40 and 80 µg/mL) on three different days. The % RSD range was obtained as 0.32-0.40 and 0.57-0.64 for intra-day and inter-day precision studies respectively (Table 3).

Table 3: Intra-day and inter-day precision studies of Rufinamide

Sample No.	Conc. (µg/mL)	Intra-day precision	% RSD*	Inter-day precision	% RSD*
		Mean* ± SD		Mean* ± SD	
1	20	2363967.67 ± 8872.07	0.38	2363353±15026.35	0.64
2	40	4654783.67 ± 14571.25	0.32	4660375±26331.69	0.57
3	80	9024309.33±35772.41	0.40	9018740.33±54186.57	0.60

**Mean of three replicates*

Because the stability of standard solutions can also affect the robustness of analytical methods, the stability of standard solutions of the drug substance used in this method was tested over a long period of time. One portion of a standard solution was kept at room temperature and the other portion was stored under refrigeration at approximately 4ºC and the content of these solutions was regularly compared with that of freshly prepared solutions. No change in drug concentrations were observed for solutions stored under refrigeration. But it is recommended that the sample and standard solutions must therefore, be freshly prepared in amber colored flasks to protect from light.

Accuracy

The method accuracy was proven by the recovery test. A known amount of Rufinamide standard (10 µg/mL) was added to aliquots of samples solutions and then diluted to yield total concentrations as 18, 20 and 22 µg/mL as described in Table 4. The assay was repeated over 3 consecutive days. The resultant % RSD was 0.83 (<2.0 %) with a recovery 97.20-97.83 %.

Table 4: Accuracy - recovery study of Rufinamide by standard-addition method

Sample No.	Spiked Conc. (µg/mL)	*Measured Conc. (µg/mL)	(%) Recovery*	(%) RSD *
1	8 (80 %)	7.78	97.25	0.83
2	10 (100 %)	9.72	97.20	
3	12 (120 %)	11.74	97.83	

**Mean of three replicates*

Limit of Detection and Limit of Quantification

The LOQ and LOD were determined based on the 10 and 3.3 times the standard deviation of the response, respectively, divided by the slope of the calibration curve. The LOQ and LOD were found to be 0.0086 µg/mL and 0.0028 µg/mL respectively.

Robustness

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability for routine analysis [10]. The robustness of the method was evaluated by assaying the same sample under different analytical conditions deliberately changing from the original condition. The detection wavelength was set at 208 and 212 nm (± 2 nm), the ratio of percentage of methanol: water in the mobile phase was applied as 54:48 and 50:50 (± 2 %, V/V), the flow rate was set at 0.9 and 1.1 mL/min (± 0.1 mL/min). The results obtained from assay of the test solutions were not affected by varying the conditions and were in accordance with the results for original conditions. The % RSD value of assay determined for the same sample under original conditions and robustness conditions was less than 2.0% indicating that the developed method was robust.

Selectivity/Specificity

The specificity of the developed method was determined by injecting sample solutions (80 µg/mL) which were prepared by forcibly degrading under such stress conditions as heat, light, oxidative agent, acid and base under the proposed chromatographic conditions. The stability indicating capability of the method was established from the separation of Rufinamide peak from the degraded samples derived from the software. The degradation of Rufinamide was found to be very similar for both the tablets and standard.

Solution Stability and Mobile Phase Stability

The %RSD of the assay of Rufinamide from the solution stability and mobile phase stability experiments was within 2%. The results of the solution and mobile phase stability experiments confirm that the sample solutions and mobile phase used during the assays were stable up to 48 h at room temperature and up to 3 months at 4ºC.

Analysis of Commercial Formulations (Tablets)

The proposed method was applied to the determination of Rufinamide tablets ^{Pr}BANZEL™® and BANZEL® and the result of these assays yielded 96.74- 97.10 % respectively with RSD < 2.0 %. The result of the assay (Table 5) indicates that the method is selective for the assay of Rufinamide without interference from the excipients used in these tablets. The typical chromatograms for Rufinamide obtained from the extracted marketed formulations were shown in Figure 4a and 4b.

Table 5: Analysis of Rufinamide commercial formulation (Tablets)

Sample No.	Formulation	Labeled claim (mg)	*Amount found (mg)	*Recovery (%)
1	^{Pr}BANZEL™®	400	388.41	97.10
2	BANZEL®	400	386.98	96.74

*Mean of three replicates

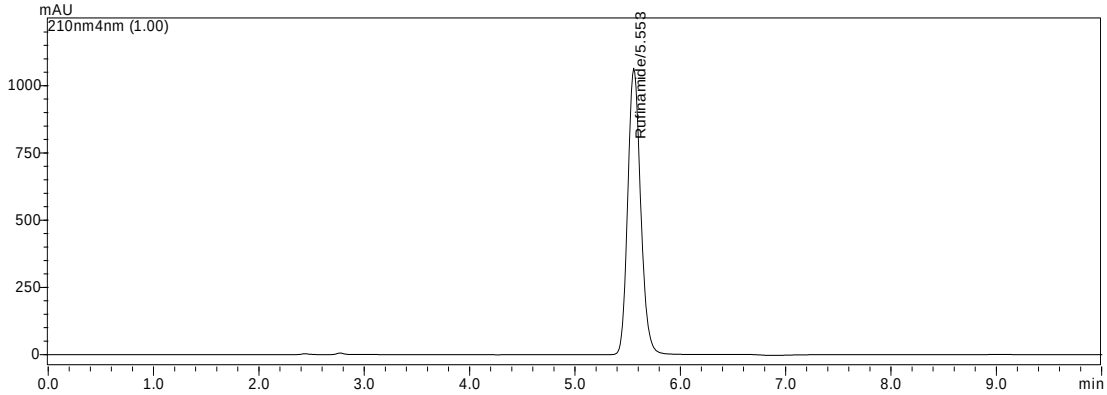

Figure 4a: Typical Chromatogram of Rufinamide (80 µg/mL) ^{Pr}BANZEL™® (400 mg)

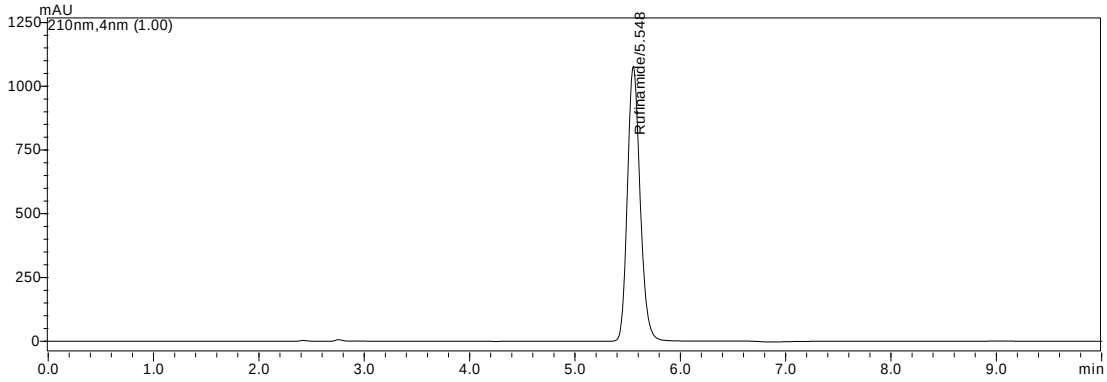

Figure 4b: Typical Chromatogram of Rufinamide (80 µg/mL) BANZEL® (400 mg)

Forced Degradation Studies

Rufinamide standard and tablet powder was found to be quite stable under dry heat conditions. Typical chromatograms obtained following the assay of stressed samples are shown in Figure 5a-5e.

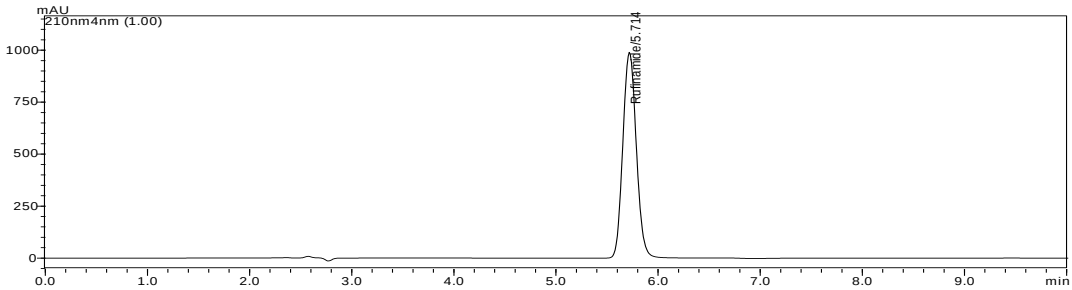

Figure 5a: Typical Chromatogram of Rufinamide (80 µg/mL) on Acidic degradation

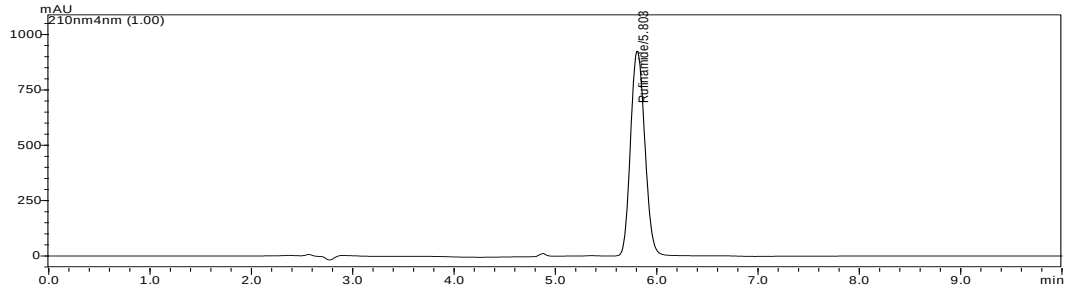

Figure 5b: Typical Chromatogram of Rufinamide (80 µg/mL) on Alkaline degradation

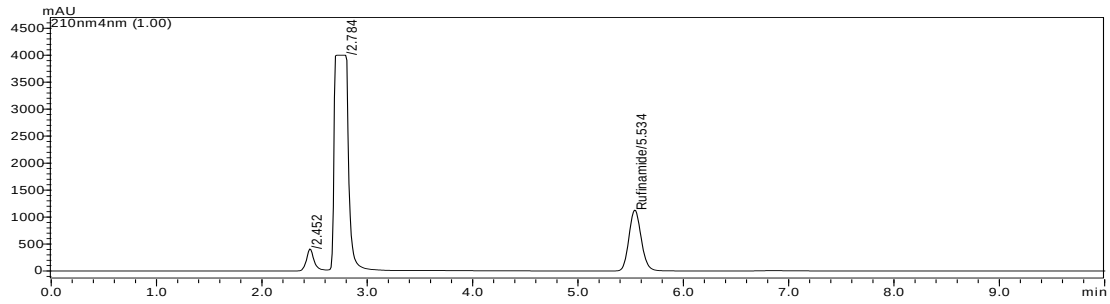

Figure 5c: Typical Chromatogram of Rufinamide (80 µg/ mL) on Oxidative degradation

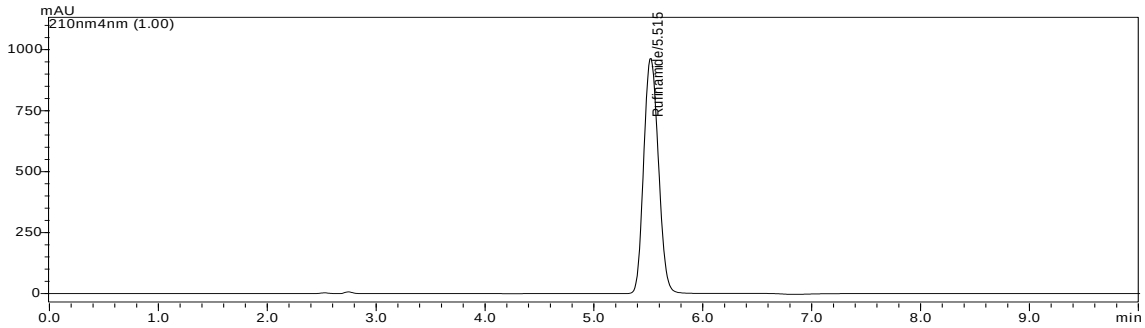

Figure 5d: Typical Chromatogram of Rufinamide (80 µg/mL) on Photolytic degradation

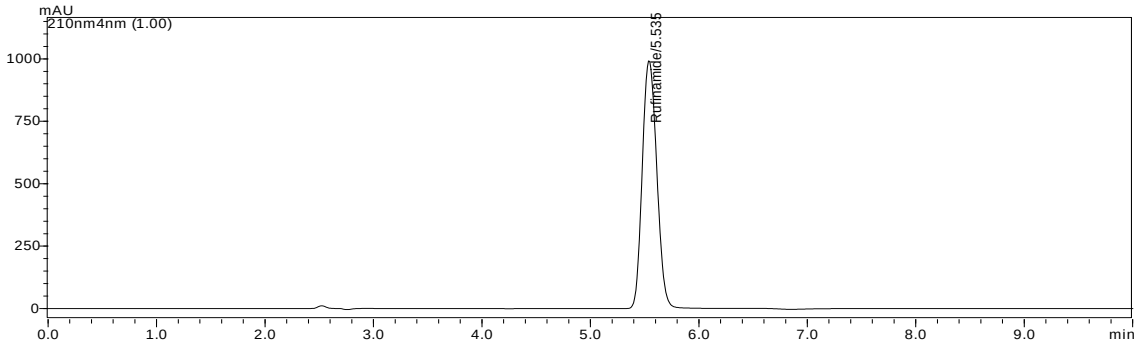

Figure 5e: Typical Chromatogram of Rufinamide (80 µg/mL) on Thermal degradation

A very slight decomposition was seen on exposure of Rufinamide drug solution to acidic (1.36), alkaline (0.58) and oxidation (0.81). During the oxidative degradation two major degradants were observed at 2.452 mins and 2.784 mins without interfering the elution of drug peak (5.534 mins) and the percentage of drug decomposition was found to be 0.81 % indicating that the drug is highly resistant towards oxidation. Rufinamide has undergone thermal (0.16) and UV degradation (0.77) very slightly i.e less than 1.0 %. Table 6 summarises the data of degradation studies.

Table 6: Forced degradation studies of Rufinamide

Stress Conditions	*Drug recovered (%)	*Drug decomposed (%)
Standard Drug	100	-
Acidic Hydrolysis	98.64	1.36
Alkaline Hydrolysis	99.42	0.58
Oxidative degradation	99.19	0.81
Thermal degradation	99.84	0.16
Photolytic degradation	99.23	0.77

Mean of three replicates

CONCLUSION

The proposed stability-indicating HPLC method was validated as per ICH guidelines and applied for the determination of Rufinamide in pharmaceutical dosage forms. The method was found to be accurate, precise, robust and specific as the drug peak did not interfere with the extra peaks aroused during the forced degradation studies. At the same time the chromatographic elution step is undertaken in a short time (< 6 min). No interference from any components of pharmaceutical dosage form or degradation products and therefore the method can be successfully applied to perform long-term and accelerated stability studies of Rufinamide formulations. In conclusion, the high sensitivity, good selectivity, accuracy

and reproducibility of the proposed method is suitable for quality control analysis of complex pharmaceutical preparation containing Rufinamide. The reduction of acetonitrile consumption is one of the best solutions to the current global acetonitrile shortage and will safeguard against future risk.

ACKNOWLEDGEMENTS

The authors are grateful of M/S GITAM University, Visakhapatnam, India for providing research facilities and also to Eisai Pharmaceuticals (India) for providing the gift samples of the drug.

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