

Method development and validation for estimation of rufinamide in tablet dosage forms by RP-HPLC

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Abstract

A simple, precise, rapid and reproducible stability indicating RP -HPLC method was developed and validated for the determination of Rufinamide in pharmaceutical dosage forms. Chromatography was performed on a C₁₈ (ODS) Acetonitrile: water: Triethylamine buffer pH4.6: Methanol (70:20:10 v/v) was used as mobile phase and flow rate was adjusted to 0.8 ml/ min. The detection was carried out at 292 nm using analytical Tech. UV-Visible detector SpD-10AVP. The obtained calibration curve was linear in the concentration range of 10–50ug/ ml. The limit of detection and quantification was found to be 1.056 ug/ml and 3.09 µg/ml respectively.

Key words: Rufinamide, Reverse phase -HPLC, PDA, and Tablet dosage forms.

Access this article online	
Quick Response Code:	Website: www.innovativepublication.com
	DOI: 10.5958/2394-2797.2016.00015.0

Introduction

Rufinamide is a triazole derivative structurally unrelated to currently market antiepileptic drug. It's practically insoluble in water, slightly soluble in tetrahydrofuran, in methanol, and very slightly soluble in ethanol and in acetonitrile.²⁻³

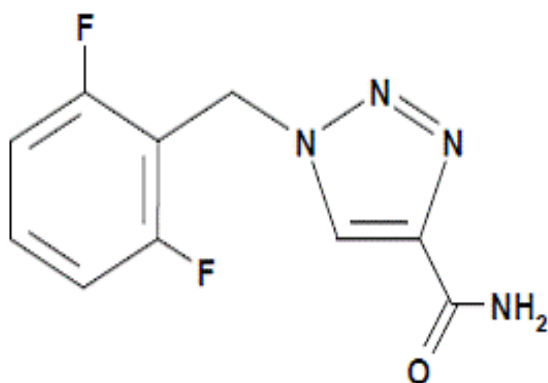


Fig. 1: Chemical Structure of Rufinamide

Literature survey revealed that numerous methods have been reported for estimation of Rufinamide in pharmaceutical formulations. Present study involves the development of HPLC method³⁻⁴ using simple mobile phase which is sensitive and rapid for quantification of Rufinamide in tablet dosage forms as well as

subsequent validation of developed method according to ICH guide lines. The important features and novelty of the proposed method included sonication of sample at ambient temperature treatment with sonication of small amount of powder sample at ambient temperature.

Experimental

All the reagents were used HPLC grade solvents for proposed method. Acetonitrile and water was purchased from SD fine Chemicals (Mumbai, India) Rufinamide sample was procure from Dr. Reddy's Labs Pvt. (Hyderabad, India). Rufinamide commercial Tablet formulation (Glenmark pharma Pvt. Ltd) was procured from local market. The tablet dosage forms obtained was containing 400 mg of Rufinamide for oral administration.

Instrumentation and analytical conditions

Waters-2695 sophisticated equipment was used for this separation process and mobile phase connecting with 2487 quaternary pump. The Analytical column C₁₈ was used and operated at 27 °C Temperature and mobile phase contains Acetonitrile: Triethylamine buffer pH4.6: Methanol (70:20:10) % v/v) was used at a flow rate of 1ml/ min. The diluents was freshly prepared and degassed by sonicating for 5 min before use. The maximum absorbance of Rufinamide was found at 243nm.

Stock solutions

Standard stock solution of 1000ug/ ml of Rufinamide was prepared freshly by accurately weighing 25mg of Rufinamide into 25ml volumetric flask.

The above solution was used for further diluted with mobile phase in 10ml volumetric flask to obtain five working standards in the concentration of 10-50

ug/ ml of Rufinamide. All the solutions were prepared in triplicates. The calibration curve was plotted with the six concentrations of the 10-50 ug/ ml working standard solutions.

Assay of Tablet formulations

The contents of twenty commercial tablets (labeled concentration 400 mg of Rufinamide) were weighed and their mean mass was determined. After grinding the tablets into a fine powder in a glass mortar, an accurately weighed quantity of the tablet powder equivalent to 25 mg of Rufinamide was quantitatively transfer into a 25 ml volumetric flask with about 20 ml of phosphate buffer pH 4.0. 1.5 ml aliquot was transferred into a 10 ml volumetric flask. The theoretical Rufinamide concentration after dilution was 30 µg/ ml (100% of Rufinamide).

Validation procedure

The proposed method validation was demonstrated that the method is suitable for its quality control dosage forms purpose as it is stated in ICH guidelines⁸. Standard plots were constructed with six concentrations in the range of 10 - 50 ug/ ml of Rufinamide prepared in triplicates to test linearity. The peak area of Rufinamide was plotted against the concentration to obtain the calibration graph. Repeatability was calculated from six replicate injections of freshly prepared Rufinamide test solution in the same equipment at a concentration of 100% of the intended test concentration value on the same day. The analyzing Rufinamide at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of Rufinamide recovered in the samples.

Results and Discussion

Optimization

Selection of the detection wavelength: The UV spectra of Rufinamide in Acetonitrile: Triethylamine buffer pH4.6: Methanol (70:20:10 v/v) mixtures of phosphate buffer and Acetonitrile in the region between 200 and 400 nm. It shows that at 292 nm, Rufinamide have maximum absorbance. Hence λ_{max} of Rufinamide in mobile phase was selected as an optimum detection wavelength for the quantification of Rufinamide.

Chromatographic Conditions

Optimized Method Parameters:

Optimized Conditions:

Mobile phase ratio: Acetonitrile: Triethylamine buffer pH4.6: Methanol (70:20:10)

Column: ODS C₁₈ (4.6×250 mm) 5µ

Column temp.: 27°C

Wavelength: 243nm

Flow rate: 0.8 ml/min

Injection vol.: 10µl

Run time: 5 minutes

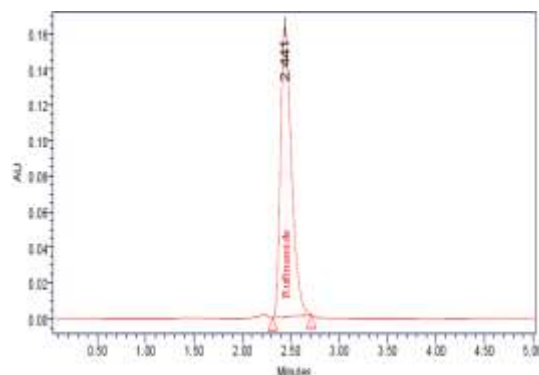


Fig. 2: Rufinamide Optimization of chromatogram

Validation of methods

Range and Linearity: Six point's calibration graphs were constructed covering a concentration range 10-50ug/ ml (Three independent determinations were performed at each concentration. Linear relationships between the of peak area signal of Rufinamide & the corresponding drug concentration was observed as shown in Fig. 3. The linearity values was (r^2) 0.9999 shown below

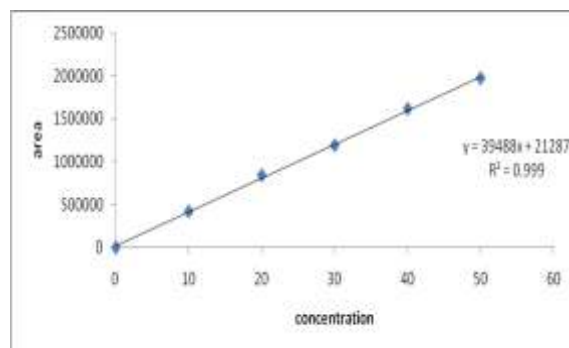


Fig. 3: Calibration Graph of Rufinamide

Precision

The validated method was applied for the assay of commercial tablets containing 4 mg of Rufinamide. Sample was analyzed for six times after extracting the drug similar procedure and assay sample preparation of the experimental section for showing that the content of Rufinamide in tablet formulations confirmed to the content requirements (95 - 105%) of the label claim.

For the intermediate precision a study was carried out by the same analyst working on the same day and on three consecutive days ($n=3$) indicated a R.S.D. of 0.0455 and 0.03995% respectively.

Accuracy

The data for accuracy were expressed in terms of percentage recoveries of Rufinamide in the real samples. (Table 4) The average recovery of Rufinamide in real sample were within the range of 99.60 and

100.05%. Mean % R.S.D. was 0.6069%, satisfying the acceptance criteria for the study.

Table 1: Accuracy study for Rufinamide (n = 9)

S. No	Percentage	% recovery	Mean \pm S.D	% RSD	S.E
1	50%	99.60	99.05 \pm 0.6211	0.72 69	0.4196
2	100%	100.05			
3	150%	99.00			

* Mean of three observations

System suitability

All system suitability parameters were represented below Table 2.

Table 2: Summary for RP-HPLC Method

Parameter	Acceptance criteria	Results obtained
		For Rufinamide
System suitability	Theoretical Plates- NLT 2000	5342
	Tailing factor- NMT 2	1.09
	Retention time	2.41
Precision	%RSD- NLT 2	0.77
Linearity	Correlation Coefficient	0.9997
Accuracy	% Recovery	100.34%
Limit of detection		1.32 μ g/ml
LOQ		3.02 μ g/ml

The proposed method accurate, reproducibility and validation parameters for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of Rufinamide in tablet formulation.

Forced Degradation Studies

Sample Preparations to Perform Forced Degradation Studies

Acid degradation: Accurately weighed 10mg of equivalent weight of Rufinamide sample into a 10ml clean dry volumetric flask and added about 3mL of 0.1N HCl

Further pipetted 0.3 ml of above stock solution into a 10ml volumetric flask (it contains Rufinamide) and diluted up to the mark with diluent.

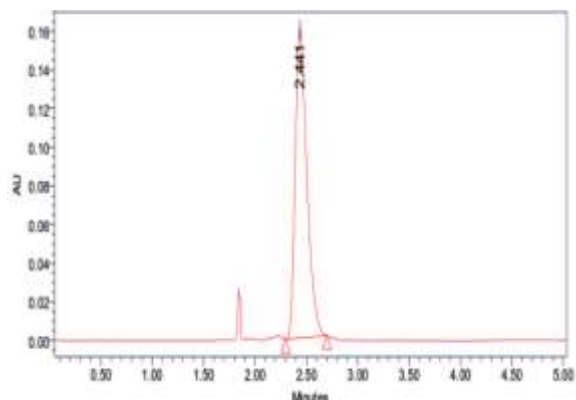


Fig. 4: Showing acid degradation for Rufinamide

Alkaline degradation

Accurately weighed 10 mg equivalent weight of Rufinamide sample into a 10mL clean dry volumetric flask and added about 1 mL of 0.1N NaOH.

Further pipetted 0.3ml of above stock solution into a 10ml volumetric flask (it contains Rufinamide) and diluted up to the mark with diluent.

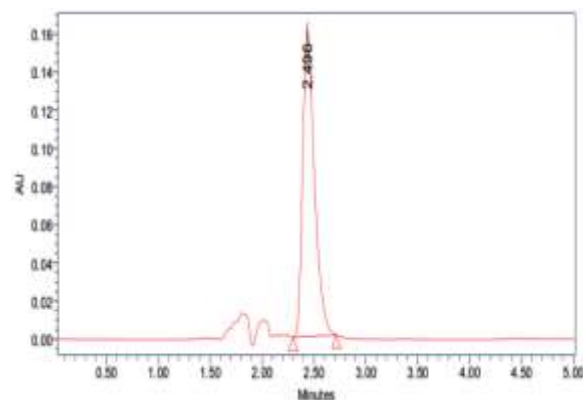


Fig. 5: Showing Alkaline degradation for Rufinamide

Peroxide degradation

Accurately weighed 10 mg equivalent weight of Rufinamide sample into a 10mL clean dry volumetric flask and added about 3mL of Hydrogen peroxide solution and kept side for 3hours and made the volume up to mark by using Diluent and sonicated to dissolve it completely.

Further pipetted 0.3ml of above stock solution into a 10ml volumetric flask (it contains Rufinamide) and diluted up to the mark with diluents Fig. 5.

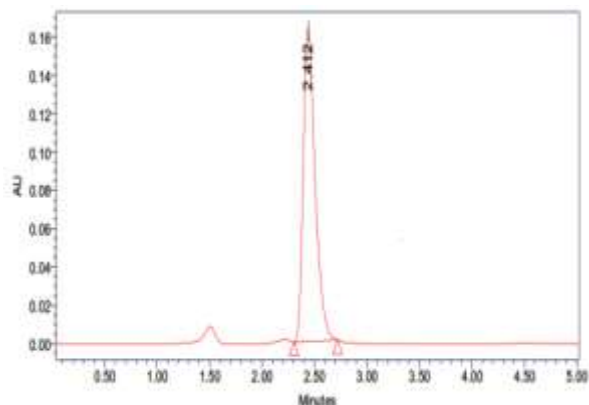


Fig. 5: Showing peroxide degradation for Rufinamide

Thermal degradation

Accurately weighed 10 mg equivalent weight of Rufinamide sample into a 10mL clean dry volumetric flask and exposed to heat at 80-90°C for 3 hours and then made the volume up to mark by using Diluent and sonicated to dissolve it completely.

Further pipetted 0.3ml of above stock solution into a 10ml volumetric flask (it contains Rufinamide) and diluted up to the mark with diluent. Fig. 6.

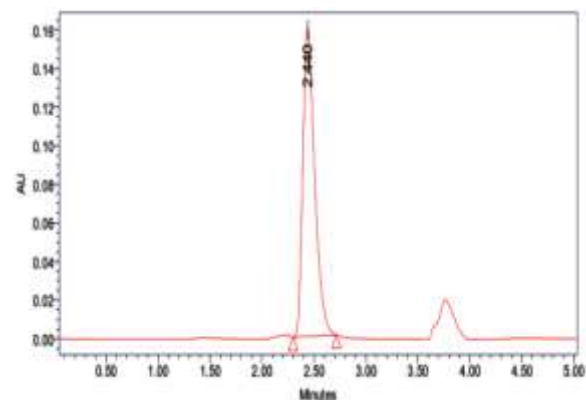


Fig. 6: Showing Thermal degradation for Rufinamide

Photolytic degradation

Accurately weighed 10 mg equivalent weight of Rufinamide sample into a 10mL clean dry volumetric flask and exposed to sunlight for 3 hours and made the volume up to mark by using Diluent and sonicated to dissolve it completely.

Further pipetted 0.3ml of above stock solution into a 10ml volumetric flask (it contains Rufinamide) and diluted up to the mark with diluent. Fig. 7

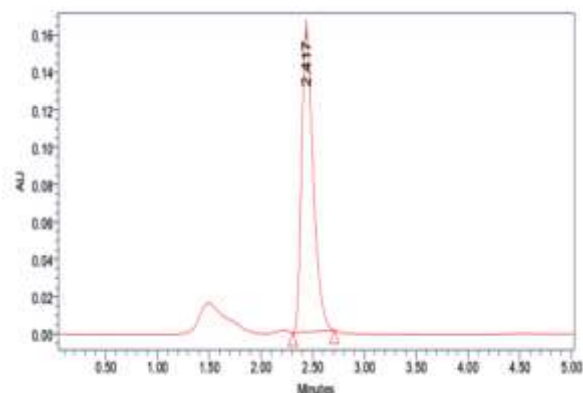


Fig. 7: Showing Photolytic degradation for Rufinamide

Table 3: Results for degradation studies

S. No	Type of degradation	Concentration of sample ($\mu\text{g/ml}$)	Area of sample	Assay content (% w/w)
1	Acid (0.1N HCl)	30 $\mu\text{g/ml}$	1206721	100%
2	Base (0.1N NaOH)	30 $\mu\text{g/ml}$	1214352	100%
3	Peroxide (3% H_2O_2)	30 $\mu\text{g/ml}$	1145269	94%
4	Thermal (at 60 ⁰ c)	30 $\mu\text{g/ml}$	1195615	99%
5	Photolytic (sunlight)	30 $\mu\text{g/ml}$	1185917	98%

Summary and Conclusion

RP-HPLC method was developed for estimation of Rufinamide in bulk and Pharmaceutical dosage form. The separation was achieved on ODS C18 (4.6 \times 250mm) 5 μ containing the mobile phase mixture of Acetonitrile: Triethylamine buffer pH4.6: Methanol (70:20:10).

Conclusion

The proposed work was specific, accurate, rapid and economical for estimation of Rufinamide in bulk and in its Pharmaceutical dosage form. The sample recoveries in all formulations were in good agreement with their respective Label Claims and the % RSD values were within 2 and the method was found to be precise. This method can be used for routine determination of Rufinamide in bulk and in Pharmaceutical dosage forms.

Acknowledgement

The authors are also thankful to the Principal and Management of Care college of Pharmacy-Warangal, for supporting entire research work.

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