INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF RUFINAMIDE IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, reproducible and efficient high performance liquid chromatographic method was developed for determination of Rufinamide in its pure form as well as in tablet dosage form. A enable pack ODS C_{18} column 250X4.6 mm 5µm particle size in isocratic mode with mobile phase containing Acetonitrile: Buffer (potassium dihydrogen phosphate) (30:70 v/v) adjusted to pH 4.5 using ortho phosphoric acid. The flow rate was 1.0 ml/min and effluent was monitored at 210 nm. The retention time and linearity range for Rufinamide was 3.18min and10-60µg/ml respectively. The developed method was found to be accurate, precise and selective for determination of Rufinamide in tablet dosage form.

Keywords: Rufinamide, RP-HPLC, Validation and Linearity.

1. INTRODUCTION

Rufinamide is an antiepileptic drug approved by the US Food and Drug Administration 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].Rufinamide is chemically known as 1-[(2, 6- difluorophenyl) methyl]-1 H-1, 2, 3-triazole-4 carboxamide (Fig. 1). The mechanism of action of Rufinamide involves 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]. To date, all analytical methods described in literature for the determination of Rufinamide in biological fluids involve liquid chromatography[3–7], liquid chromatography–mass spectrometry[8], Uv-Spectro photometric[9] and HPLC[10-13] methods. In the present work, we developed a simple, precise, accurate, selective and robust liquid chromatographic method for the determination of Rufinamide in pharmaceutical dosage form as an alternative method.

2. EXPERIMENTAL

Chemicals and Reagents

Rufinamide standard procured as gift sample from Hetero drugs Limited (Hyderabad, India). Acetonitrile HPLC grade, Methanol HPLC grade,(Rankem,Mumbai, India), Potassium dihydrogen phosphate purchased from (E.Merck Mumbai, India), and ortho phosphoric acid (SD Fine Chemicals, Ahmadabad, India), All chemicals were of analytical grade and used as received. Rufinamide is available as tablets with brand names PrBANZEL™ and BANZEL®with label claim of100, 200 and 400mg of drug.

HPLC instrumentation and Chromatographic conditions

A prominence isocratic HPLC system (SHIMADZU High performance liquid chromatography with UV detector) enable column C18 (250x4.6 mm, 5µm). A 20µL Rheodyne injection syringe was used for

sample injection. HPLC grade, Acetonitrile, Water and AR grade phosphate buffer were used for the preparing the mobile phase. A freshly prepared, Acetonitrile: Buffer (PH-4.5) (30:70 v /v) was used as the mobile phase. The solvents was filtered through a 0.45µ membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1 ml/min, column temperature was maintained at room temperature and the detection of the drug was carried out at 210nm.

Preparation of buffer

Weigh 13.690 grams of Potassium dihydrogen phosphate (0.1M) into a 1000ml beaker, dissolve and diluted to 1000ml with HPLC water.Adjusted the pH of buffer to 4.5 with orthophosphoric acid.

Preparation of mobile phase

Mix a mixture of above buffer 700 ml (70%) and 300 ml of Acetonitrile (30%) and Filter through 0.45 µ filter under vacuum filtration. Degas in ultrasonic water bath for 15 minutes.

Diluent Preparation

Mobile phase as diluents

Standard Solution Preparation

Accurately weighed and transferred 10mg of Rufinamide working standard into a 10 ml volumetric flask added about 7 ml of Methanol and sonicated to dissolve it completely and make volume up to the mark with the diluent. (Stock solution) Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filtered through 0.45µm filter.

Sample solution preparation

Weigh and powder about twenty tablets in a neat clean and dry motor and pestle grind and mix to uniform powder. Accurately weigh and transfer the sample equivalent to 10 mg of Rufinamide into a 10 ml volumetric flask, add about 5 ml of methanol and sonicated for 5 minutes, and diluted to volume with mobile phase. filter through 0.4 µ membrane filter, from the filtrate pipette out 0.5ml of sample solution into a 10 ml volumetric flask, make up the volume with diluent (mobile phase) and sonicated for 5 minutes.

UV Spectra of Rufinamide

An absorbance maximum of Rufinamide was detected at 210 nm.The drug showed linearity with absorbance in the range 10-60 µg/ml, when measured at 210 nm. Calibration curves were plotted from the absorbance values at this wavelength. It has shown in **Fig: 2.**

3. METHOD VALIDATION

The method was validated for the following parameters: Linearity, Precision, Accuracy, Limit of Quantitation (LOQ), Limit of Detection (LOD), Robustness, and System Suitability. [14]

Linearity

The linearity of the method was demonstrated over the concentration range of 10- 60µg / ml of the target concentration. Aliquots of 10, 20, 30, 40, 50 and 60 µg/ml were prepared from above prepared stock solution. Different concentrations of the pure drug were injected into the chromatographic system. Calibration curve of Rufinamide was constructed by plotting peak area vs. applied concentration of Rufinamide.The obtained results shown an excellent correlation between peak area and concentration of pure drug within the concentration range & it has shown in **Fig: 3.** The correlation coefficient for the average area at each level versus concentration of analyte was calculated and is presented in **Table: 1.**

Method Precision

The precision of the method was demonstrated by six repeated injections of standard solution (100%) was made and the response factor of drug peak and % RSD were calculated and present in **Table:2.** From the data obtained, the developed method was found to be precise.

Accuracy

A Study of recovery of Rufinamide from spiked placebo was conducted at three different spike levels i.e.50, 100 and 150 Samples were prepared with Rufinamide raw material equivalent to about the target initial concentration of Rufinamide. Sample solutions were prepared in triplicate for each spike level and assayed as per proposed method. The % recovery was given in **Table-3.** The mean recoveries of Rufinamide from spiked were found to be in the range of 98.62- 99.98%.

Limit of Quantitation (LOQ) and Limit of Detection (LOD)

The LOQ and LOD were based on the standard deviation of the response and the slope of the constructed calibration curve (n¼3),as described in International Conference on Harmonization guidelinesQ2(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= 3.3× Standard Deviation of Y-Intercept **/** Slope of the Calibration Curve
LOQ=10× Standard Deviation of Y-Intercept **/** 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. LOD and LOQ were calculated and presented in **Table: 4.**

Robustness
The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (210 and 212 nm), percentage of Acetonitirile in the mobile phase (30and32), pH of the buffer (4.5and 4.6) and flow rate (1.0 and 1.1ml/min).Robustness of the method was studied using six replicates at a concentration level of 40 µg /ml of Rufinamide.

System suitability
System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (RT), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 50µg/ ml.The results given in **Table: 5.** were within acceptable limits.

Assay of the tablet dosage form (Rufinamide 400mg/tablet)
The proposed validated method was successfully applied to determine Rufinamide (BANZEL®) in tablet dosage form. The result obtained for Rufinamide was comparable with corresponding labeled amount **(Table 6).**

CONCLUSION
The developed validated RP-HPLC method for determination of Rufinamide in bulk and in its pharmaceutical dosage form, it was found to be simple, precise and rapid. The assay result obtained by this method is in fair agreement. This method can be used for the routine determination of Rufinamide in bulk and its commercial formulations.

ACKNOWLEDGMENTS
The authors are grateful to Management of Anurag Pharmacy College (Ananthagiri, Kodad, and Nalgonda, India) for providing research facilities during course of this work and also to Hetero Drugs Limited (Hyderabad, India) for providing the gift sample of the drug. And special thanks to Dr.M.Chinna Eswaraiah, principal of Anurag Pharmacy College for his valuable guidance and constant encouragement to complete this work.

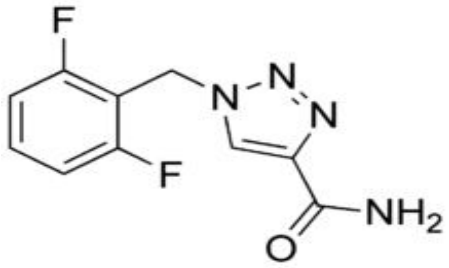

Fig. 1: Chemical structure of Rufinamide

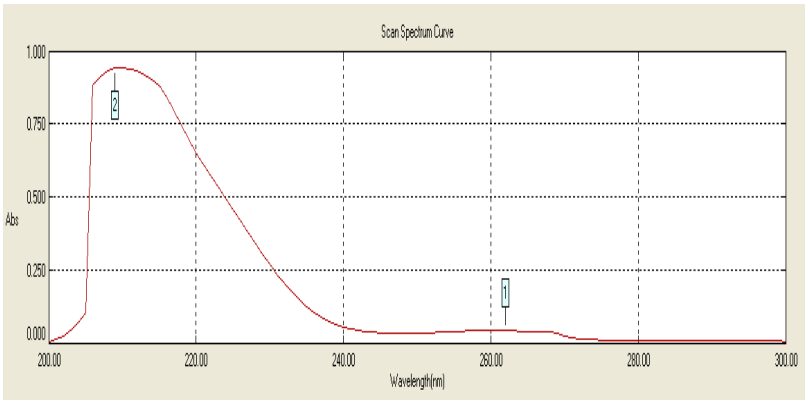

Fig. 2: UV Spectra of Rufinamide

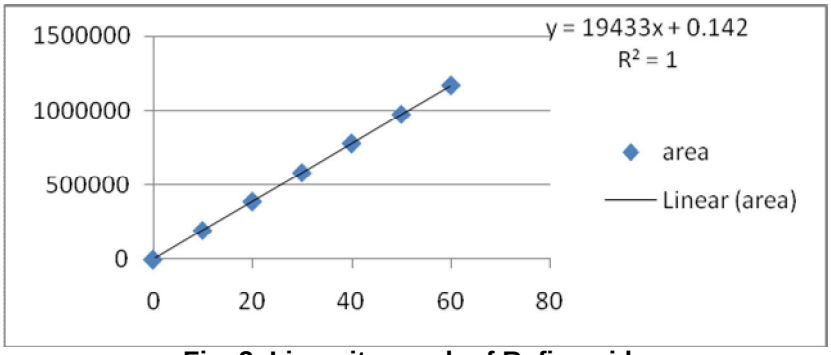

Fig. 3: Linearity graph of Rufinamide

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Sample Name:RUFINAMIDE
Sample ID:LIN_10

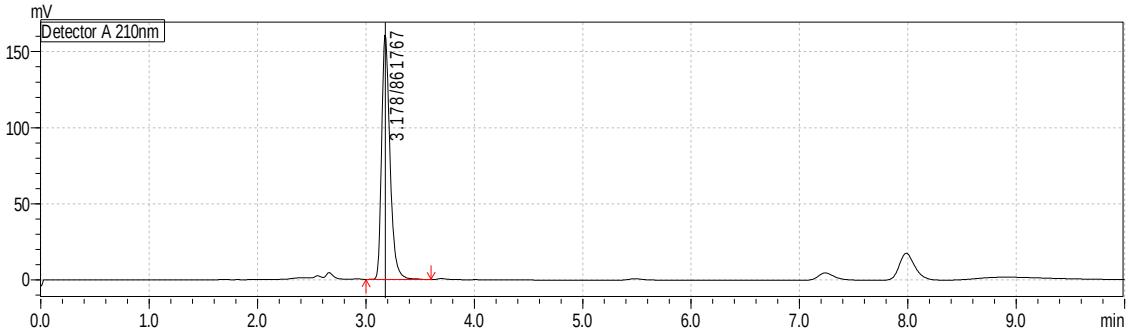

Fig. 4: Chromatogram of Rufinamide at 210 nm

Table 1: Linearity results for Rufinamide

S.No	% of Test	Concentration (µg/ml)	Area
1	25	10	194325
2	50	20	388650
3	75	30	582976
4	100	40	777300
5	125	50	971625
6	150	60	1165950

Table 2: Precision results of Rufinamide

S.No	Retention Time(min)	Area
1	3.18	777302
2	3.17	777300
3	3.17	777298
4	3.18	777294
5	3.19	777298
6	3.18	777299
Average	3.178	777298.5
S.D	0.00752	2.664583
%R.S.D	**0.2366**	**0.000342**

Table 3: Accuracy results of Rufinamide

S.No	Spike Level	µg/ml added	µg/ml found	% recovery	mean % recovery
1	50%	20	19.64	98.24	98.64%
2	50%	20	19.90	99.52	
3	50%	20	19.64	98.24	
1	100%	40	40	100	99.99%
2	100%	40	39.99	99.97	
3	100%	40	40	100	
1	150%	60	59.99	99.98	99.98%
2	150%	60	59.99	99.98	
3	150%	60	59.99	99.98	

Table 4: LOD and LOQ results of Rufinamide

Parameter	Rufinamide
LOD	1.52µg/ml
LOQ	4.39 µg/ml

Table 5: System suitability studies of Rufinamide by RP-HPLC method

Parameter	Acceptance criteria	Observed value
1. Retention time (RT)	RSD ≤ 1%	3.18
2.Theoritical plates (N)	(Not Less Than 3000)	3204
3.Tailing factor (T)	(Not More Than 2)	1.2
4. Resolution(Rs)	(Rs>2)	---

Table 6: Analysis of Rufinamide commercial formulation (Tablets)

Drug	%Assay	Amount Present
Rufinamide	98.24	392.98mg/tab

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