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

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Rufinamide, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Symmetry C18 (4.6 x 150mm, 5 µm) column using a mixture of, Methanol and Water (50:50 v/v) as the mobile phase at a flow rate of 1 mL/min, the detection was carried out at 220nm. The retention time of the drug was 5.20±0.02 min. The method produced linear responses in the concentration range of 10-60 mg/ml of Rufinamide. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of Bulk and pharmaceutical formulations.

Key words: Rufinamide, RP-HPLC, Validation.

INTRODUCTION:

Rufinamide [1,2] is a triazole derivative specifically synthesized as an anticonvulsant drug. It is chemically 1-(2, 6-difluorobenzyl)-1H-1,2,3-triazole-4-carboxamide. It is used in combination with other medication and therapy to treat Lennox–Gastaut syndrome and various other seizure disorders. A literature survey reveals that few liquid chromatography procedures have been reported for the determination of Rufinamide [3-6]. The author have developed a liquid chromatographic and validated, sensitive and reproducible method for the determination of Rufinamide in Bulk and pharmaceutical dosage forms.

EXPERIMENTAL WORK [7,8]

MATERIALS AND METHODS

Chromatographic conditions

A prominence isocratic HPLC system (Agilent High performance liquid chromatography with Auto Sampler and UV detector) Qualisil gold column C18 (4.6 x 150mm, 5 µm). A 20µL Rheodyne injection syringe was used for sample injection. HPLC grade, Methnol and Water were used for the preparing the mobile phase. A freshly prepared, Methanol:Water (P^H -3) (50 : 50 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 220nm.

Preparation of buffer

Weigh 7.0 grams of Potassium di hydrogen phosphate into a 1000ml beaker, dissolve and diluted to 1000ml with HPLC water. Adjusted the pH of water to 3 with Orthophosphoric acid.

Preparation of mobile phase

Mix a mixture of above water 500mL (50%) and 500 mL of Methanol HPLC (50%) and degas in ultrasonic water bath for 15 minutes. Filter through 0.45 µ filter under vacuum filtration.

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Diluent Preparation

Mobile phase as diluents

Standard Solution Preparation

Accurately weigh and transfer 10mg of Rufinamide Working standard into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (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 filter through 0.45µm filter.

Sample Solution Preparation

Weigh 5 Rufinamide Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Rufinamide into a 10 mL volumetric flask. Add about 7 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Method validation [9-13]

Linearity:

The linearity of the method was demonstrated over the concentration range of 10- 60 mcg / ml of the target concentration. Aliquots of 10, 20, 30, 40, 50 and 60 mcg / 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. A typical Chromatogram is shown in Fig: 1. The obtained results shown an excellent correlation between peak area and concentration of pure drug within the concentration range & it has shown in Fig: 2. The correlation coefficient for the average area at each level versus concentration of analyte was calculated and is presented in Table: 1. and their calibration parameters were shown in Table: 2.

Precision Method

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six repeated injections of standard solution was made and the response factor of drug peak and % RSD were calculated and present in Table: 3. The chromatogram was shown in Fig 3. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and response factor of drugs peak and % RSD were calculated shown in Table 3. 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-4. The mean recoveries of Rufinamide from spiked were found to be in the range of 99.08- 100.93%.

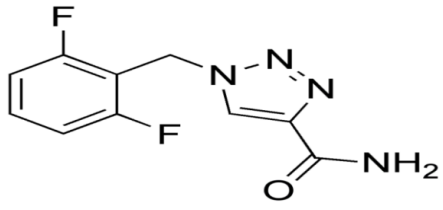
LOD and LOQ

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The residual standard deviation of the regression lines and slope of the calibration curves were used to calculate the LOD and LOQ (Table No. 2).

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 (R_t), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 100 mcg / ml. The results given in **Table: 5.** were within acceptable limits.

Chemical structure of Rufinamide



RESULTS

Table 1. Linearity results for Rufinamide

Conc. (mcg / ml)	10	20	30	40	50	60
Avg. Area	158751	314117	464005	614915	752760	890650
Correlation	0.999					

Fig: 1. Chromatogram of Rufinamide at 220 nm

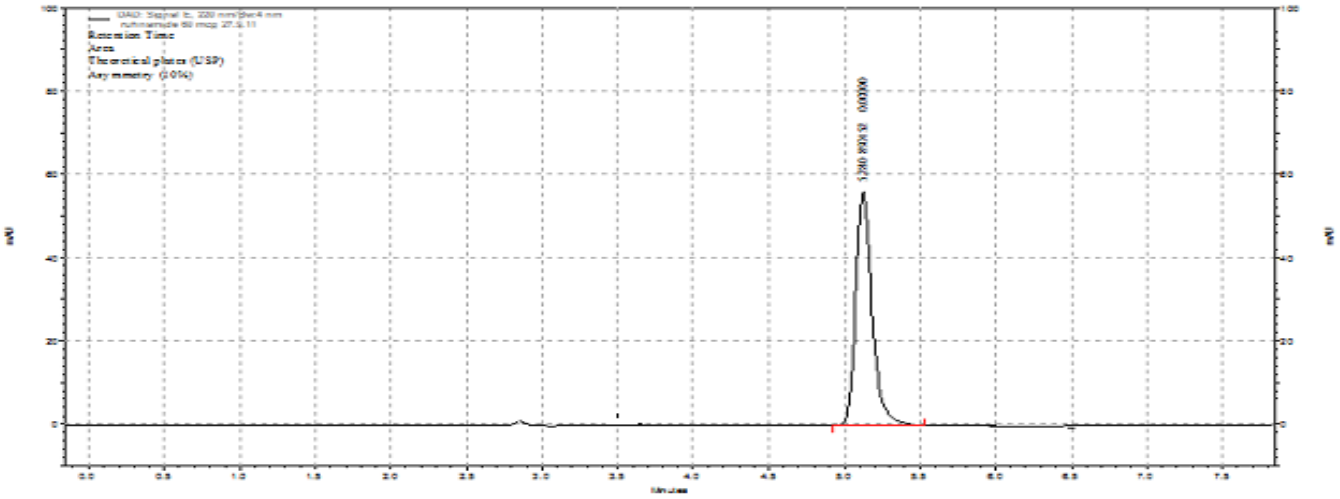


Fig: 2. Calibration curve of Rufinamide at 220 nm

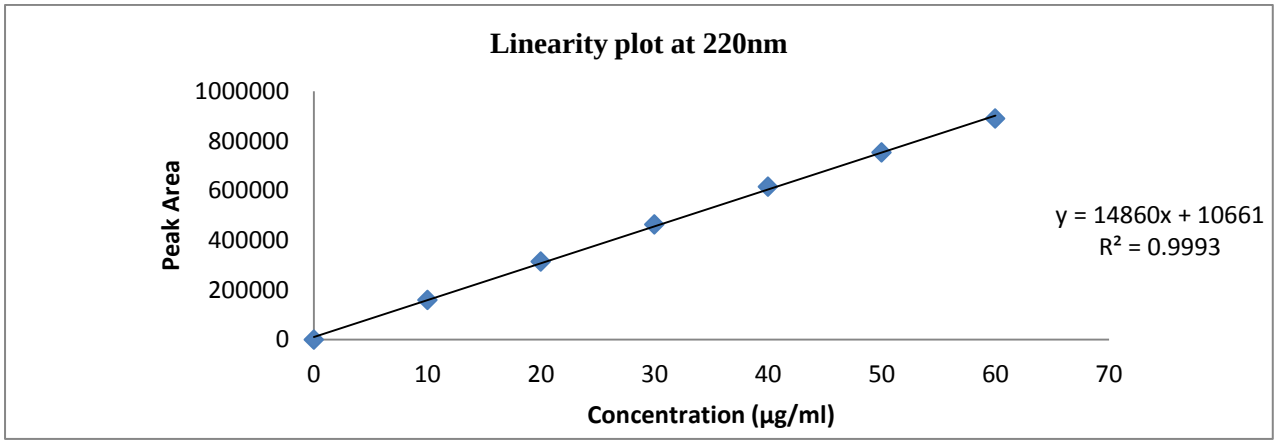


Fig 3. Chromatogram of precision

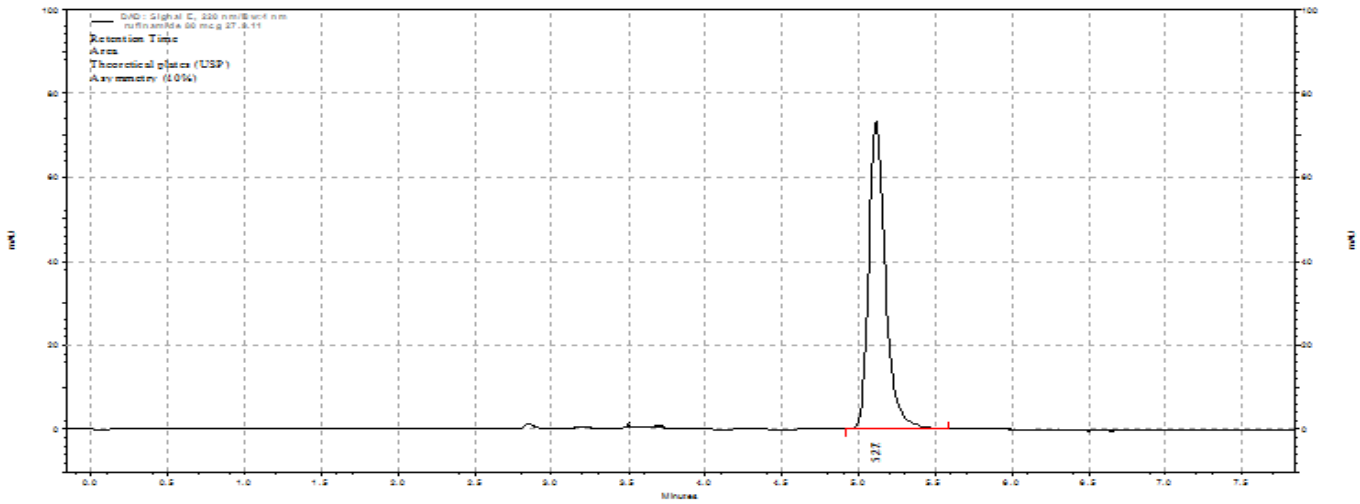


Table 2. Characteristic parameters of Rufinamide for the proposed RP-HPLC method

Parameters	RP-HPLC
Calibration range (mcg / ml)	10-60
Detection wavelength	220 nm
Mobile phase (Methanol: Water)	50:50
Retention time	5.203 ± 0.02
Regression equation (Y*)	y = 14860x + 10661
Slope (b)	14860
Intercept (a)	10661
Correlation coefficient(r^2)	0.999
Intraday Precision (% RSD*) Interday Precision (% RSD*)	1.05 1.02
Limit of detection (mcg / ml)	1.51
Limit of quantitation (mcg / ml)	4.60

Table 3. Precision results for Rufinamide

Sr. No.	Concentration (mcg / ml)	Intraday precision(Area)	Interday precision (Area)
1	40	610562	609231
2	40	614915	604938
3	40	609231	599231
4	40	599231	609231
5	40	604915	599231
6	40	599234	614915
Mean		606347.5	606129.5
Std.Dev		6368.985	6213.242
%RSD.		1.05	1.02

Table: 4. Accuracy results for Rufinamide

Sample No.	SpikeLevel	Amount (mcg / ml) added	Amount (mcg / ml) found	% Recovery	Mean % Recovery
1	50 %	20	20.10	101.58	100.93
	50 %	20	19.89	100.55	
	50 %	20	20.13	101.40	
2	100 %	40	39.79	98.91	99.08
	100 %	40	40.21	99.44	
	100 %	40	39.75	98.45	
3	150 %	60	60.13	100.37	100.28
	150 %	60	59.89	100.18	
	150 %	60	60.10	100.52	

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

Property	Values	Required limits
Retention time (R_t)	5.203 ± 0.02	RSD ≤ 1%
Theoretical plates (N)	2704	$N > 2000$
Tailing factor (T)	1.5	$T \leq 2$

DISCUSSION

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. The objective of this study was to develop a rapid and sensitive RP-HPLC method for the analysis of Rufinamide in bulk drug and pharmaceutical dosage form by using the most commonly employed RP C-18 column with UV-detection.

The run time was set at 8 min and the retention time for Rufinamide was 5.203 ± 0.2 min. Each sample was injected 5 times and the retention times were same. When the concentrations of Rufinamide and its respective peak areas were subjected to regression analysis by least squares method, a good linear relationship (r^2= 0.999) was observed between the concentration of Rufinamide and the respective peak areas in the range 10-60 mcg / ml. The regression equation was used to estimate the amount of Rufinamide, either in tablet formulations or in validation study (precision and accuracy). For the proposed RP-HPLC method, characteristic parameters were shown in Table 2.

To analyse tablet formulations, RP-HPLC method has been developed. Rufinamide tablets were analyzed as per the procedure described above. The low % RSD values (≤ 2) indicated that the method was precise and accurate. The mean recoveries were found in the range of 99.08 – 100.93 %. No interfering peaks were found in the chromatogram indicating that excipients used in the tablet formulation did not interfere with the estimation of the drug by the proposed RP-HPLC method.

The proposed RP-HPLC method was also validated for intra and inter-day variation. When the solution containing 40 mcg/ml of Rufinamide was repeatedly injected on the same day, the %RSD in the peak area for six replicate injections was found to be 1.05% Also the inter day variation (6 days and six injections) was found to be 1.02%. The results are presented in Tables 3. The % RSD values were within 2 and the method was found to be precise. It can concluded The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Rufinamide and can be reliably adopted for routine quality control analysis of Rufinamide in Bulk and its pharmaceutical formulations.

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