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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC METHOD FOR ESTIMATION OF RUFINAMIDE IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, specific, accurate and stability indicating reversed phase high performance liquid chromatographic method was developed for the determination of Rufinamide, using C18 column and a mobile phase composed of acetonitrile: water (60:40 v/v), pH 7.0. The retention time of Rufinamide was found to be 3.043 min. Linearity was established for Rufinamide in the range of 10-80 µg/mL. The percentage recovery of Rufinamide was found to be in the range of 97.15-100.6 %. The drug was subjected to acid, alkali, oxidation, dry heat and Photolytic degradation. The degradation studies indicated condition was well resolved from the pure drug with significant differences in their retention time values. This method can be successfully employed for quantitative analysis of Rufinamide in bulk drug and formulation.

KEY WORDS: Rufinamide, Stability indicating method, HPLC.

INTRODUCTION

Rufinamide is an antiepileptic drug approved by the USFDA as an adjunctive treatment for seizures associated with Lennox-gastaut syndrome under the children of 4 years of age. Principle mechanism involved in the antiepileptic activity of Rufinamide is its ability to modulate the activity of sodium channels, limiting high-frequency firing of neuronal action potentials over a broad range of concentrations. Rufinamide is a triazole derivative (Figure 1)

that is structurally distinct from currently marketed antiepileptic drug. Chemically Rufinamide is (1-[(2, 6-difluorophenyl) methyl]-1hydro-1, 2, 3-triazole-4 carboxamide).

Literature survey reveals that few UPLC, RP-HPLC in bio samples, UV spectrophotometric methods has been reported for the estimation of Rufinamide. No stability indicating RP-HPLC method for estimation of Rufinamide. The validation has been carried out as per ICH guidelines.

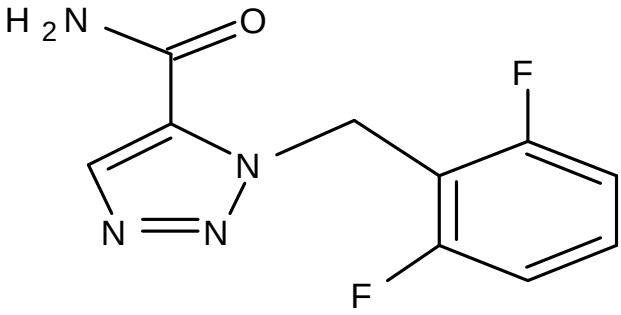

Figure 1: Rufinamide Structure

MATERIALS & METHODS

Instruments

The liquid chromatographic system was of Agilent Technology (model 1220 Infinity LC) which consisted of following components: Column C18 – phenomenex Luna (250 x 4.6 mm, 5 µ (100 A°)), UV detector, a manual injection facility with 20 µL fixed loop.

Reagents

Rufinamide bulk powder was kindly supplied as a gift sample by Torrent Research Center, Ahmadabad. Acetonitrile and water used were of HPLC grade. Banzel (200 mg) were purchased from local market.

Optimized Chromatographic Condition

A reverse phase C-18 column equilibrated with mobile phase comprising of mixture acetonitrile: water (60: 40) was used. Mobile phase flow rate was maintained 1 mL/min and eluent were monitored at 210 nm. A 20 µL sample was injected using a fixed loop, and the total run time was 7 min. all the chromatographic separations were carried out at controlled room temperature (25 °C).

Selection of Mobile Phase

Based on sample solubility, stability and suitability various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard solution was run in different

mobile phases tried, mobile phase containing acetonitrile: water (60:40 % v/v) proportion with detection wavelength 210 nm was selected, since it gave sharp peak with good symmetry within limits for the drug.

Preparation of Standard Stock solution

An accurately weighed quantity of Rufinamide (25 mg) was transferred to 25 mL volumetric flask, dissolved and diluted to the mark with acetonitrile to obtained standard stock solution having concentration of 1000 µg/mL.

Preparation of Calibration Curve

Aliquots of 0.1, 0.2, 0.3, 0.4, 0.6, 0.8 mL standard stock solution (1000 µg/mL) was transferred to the 10 mL of volumetric flask and made up to the mark with acetonitrile to get concentration of 10, 20, 30, 40, 60, 80µg/mL. An aliquot (20 mL) of each solution was injected under the operating chromatographic condition as described above and response were recorded. Calibration curve was constructed by plotting the peak areas versus the concentration and the regression equation was calculated.

Method Validation

The optimized Chromatographic method was completely validated according to the procedures described in ICH guidelines Q2 (R1) for the validation of analytical method.

Linearity

Appropriate aliquots of Rufinamide stock solutions were taken in different 10 mL volumetric flasks and diluted up to the mark with water to obtain final concentrations of 10-80 µg/mL of Rufinamide. The solutions were injected and chromatograms were recorded. Calibration curves were constructed by plotting average peak area versus concentrations and regression equations were computed for the drug (Figure 2 & Table 1).

Intermediate Precision

Intraday and interday precision study of Rufinamide was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for the concentration of Rufinamide (10, 40 & 80 µg/mL). The results were reported in terms of relative standard deviation (RSD) (Table 2 & 3).

Method Precision (Repeatability)

The Precision was checked by repeated measurement of the response of solutions (n=6) of Rufinamide (10 µg/mL) without changing the parameters. (Table 4)

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) of drug were derived by calculating the signal to noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations designated by ICH guideline

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ =the standard deviation of the response

and S= slope of the calibration curve.

Accuracy (Recovery Study)

The accuracy of the method was determined by calculating percentage recovery of Rufinamide. For drug, recovery studies was carried out by applying the method to drug sample to which known amount of Rufinamide corresponding to 50, 100 and 150 % of label claim had been added (standard addition method). At each level of the amount six determinations were performed and the results obtained were compared. (Table 5)

Robustness

For robustness evaluation of HPLC method a few parameters like flow rate and composition of mobile phase were deliberately changed. One factor was changed at one time to estimate the effect. Each factor selected was changed at three levels (-1, 0, +1) with respect to optimized parameters. Robustness of the method was done at the concentration level 20 µg/mL for Rufinamide (Table 6)

Solution Stability

The solution stability of Rufinamide was investigated by leaving sample in tightly capped volumetric flask at room temperature for 72 hrs and determined every 24 hr up to the study period.

System Suitability

System suitability test are an integral part of chromatographic method which are used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain

system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level 20 µg/mL for Rufinamide to check the reproducibility of the system and the results are shown in table 7.

Analysis of Marketed Formulation

Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 200 mg Rufinamide was weighed accurately and transferred to a 100 mL volumetric flask containing 50 mL acetonitrile, and sonicated for 15 minutes. Solution was filtered through Millipore 0.22 µm filter and volume was made up to the mark. Aliquot (1mL) was taken and transferred to 10 mL volumetric flask and volume was made upto the mark with acetonitrile to give a solution containing 0.2 mg /mL Rufinamide. From this solution 1 mL solution was transferred to 10 mL volumetric flask and volume was made upto the mark with mobile phase to give a solution containing 20 µg/mL Rufinamide. 20 µL was injected and the amount of Rufinamide was calculated from the regression equation. (Table 8)

Forced Degradation studies

Rufinamide was subjected to various forced degradation conditions to effect partial degradation of the drug preferably in 20-80 % range. The study provides information about the conditions in which the drug is unstable so that measures can be taken during formulation to avoid potential instabilities.

A forced degradation study of the drug was carried out under conditions of acid and base hydrolysis, photolytic, dry heat and oxidation.

Acid Degradation

Accurately weighed Rufinamide (100 mg) was transferred into 100 mL volumetric flask, 50 mL methanol and 50 mL 1N HCl was added to it. The drug was subjected to accelerated degradation at 80° C for a period of 3 hours. The accelerated degradation in acidic media was performed in the dark in order to exclude the possible effects of light on the drug. From the resultant solution, 1 mL was transferred into 50 mL flask, neutralized with 1 N NaOH and diluted upto the mark with methanol. 20 µL of the sample was injected in HPLC system and analyzed.

Base degradation

Accurately weighed Rufinamide (100 mg) was transferred into 100 mL volumetric flask, 50 mL methanol and 50 mL 1N NaOH was added to it. The drug was subjected to accelerated

degradation at 80° C for a period of 3 hours. The accelerated degradation in basic media was performed in the dark in order to exclude the possible effects of light on the drug. From the resultant solution, 1 mL was transferred into 50 mL flask, neutralized with 1 N HCl and diluted upto the mark with methanol. 20 µL of the sample was injected in HPLC system and analyzed.

Hydrogen peroxide induced degradation (Oxidation)

Accurately weighed Rufinamide (100 mg) was transferred into 100 mL volumetric flask, 50 mL methanol and 50 mL 3 % v/v hydrogen peroxide was added to it. The solution was kept in dark for 24 h at room temperature. The solution was heated after 24 h in boiling water bath for 1 h till the removal of excess hydrogen peroxide. From the resultant solution, 1 mL was transferred into 50 mL flask, diluted upto the mark with methanol and 20 µL of the sample was injected in HPLC system and analyzed

Photochemical degradation

Accurately weighed Rufinamide (100 mg) was taken into 100 mL volumetric flask and dissolved in and diluted upto the 100 mL with methanol. The photochemical stability of the drug was studied by exposing this drug solution to direct sunlight for 48 hours. Aliquot (1 mL) was transferred into 50 mL volumetric flask and diluted upto the mark with methanol. 20 µL of the sample was injected in HPLC system and analyzed

Dry heat degradation (Thermal degradation)

Rufinamide (100 mg) was stored at 100 ˚C for 12 hours under dry heat condition. After 12 hours the drug sample was dissolved in and diluted to 100 mL with methanol Aliquot (1 mL) was transferred into 50 mL volumetric flask and diluted upto the mark with methanol. 20 µL of the sample was injected in HPLC system and analyzed

RESULTS AND DISCUSSION

The mobile phase consisting of Acetonitrile: Water (60:40 v/v) pH 7.0, at 1 mL/min flow rate was optimized which gave sharp, well resolved peaks for Rufinamide. Quantification was achieved with UV detection at 210 nm based on peak area. The retention time was found to be 3.047 min. The optimized method was validated as per ICH guidelines. The drug has shown more degradation in acid hydrolysis and base hydrolysis. It was less degraded in photolytic, oxidative and thermal degradation. The absence of interference peak indicates that method can be used for routine analysis of Rufinamide in pharmaceutical dosage form.

Table 1: Linearity data

Concentration (µg/mL)	Area 1	Area 2	Area 3	Average Area	SD	CV
10	17395779	17405468	17391225	17397491	7274.14	0.04
20	35657744	35719821	34879654	35419073	468180.60	1.32
30	51652351	50564421	50987599	51068124	548416.90	1.07
40	69000972	68999782	69158632	69053129	91370.50	0.13
60	102591322	103513176	102512681	102872393	556325.70	0.54
80	137237740	135439712	137124450	136600634	1006982.00	0.73

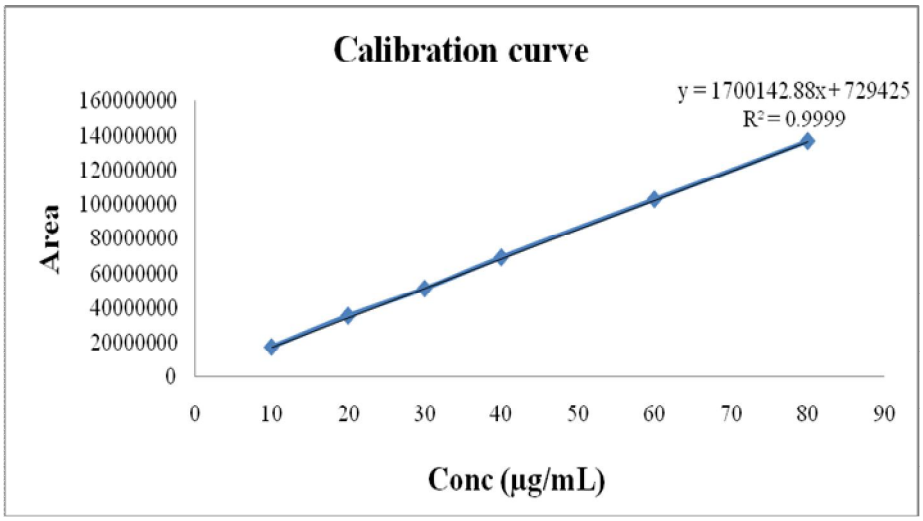

Figure 2: Calibration curve

Table 2: Intraday Precision data

concentration (µg/mL)	Area 1	Area 2	Area 3	Average area	SD	CV
10	17395779	17405468	17391225	17397490.67	7274.14	0.04
40	69000972	68999782	69158632	69053128.67	91370.50	0.13
80	137237740	135439712	137124450	136600634	1006982.00	0.73

Table 3: Interday precision data

Concentration (µg/mL)	Area 1	Area 2	Area 3	Average area	SD	CV
10	17395779	16968579	17426666	17263674.67	256026.50	1.48
40	69000972	69102450	69002456	69035292.67	58164.69	0.08
80	137237740	137098741	139121204	137819228.30	1129684.00	0.81

Table 4: Repeatability data

Sr No	Area
1	17395779
2	17402143
3	17387654
4	17395564
5	16989875
6	17378649
mean	17324944
SD	164348.7
CV	0.94

Table 5: Accuracy data

Level	Amount Added (µg)	Total Amount (µg)	Amount Recovered (µg)	% Recovery
0		20	19.89	99.45
50	10	30	29.95	100.6
100	20	40	39.32	97.15
150	30	50	49.68	99.3

Table 6: Robustness data

Sr. No.	Parameters	Retention time	Asymmetry factor	% RSD of response
1.	Acetonitrile: water (60:40 v/v)	3.047	0.95	0.24
2.	Acetonitrile: water (62:38 v/v)	2.990	0.98	0.31
3.	Acetonitrile: water (58:42 v/v)	3.208	0.89	0.24
4.	Flow rate (1mL/min)	3.047	0.95	0.24
5.	Flow rate (0.9mL/min)	3.154	0.95	0.23
6.	Flow rate (1.1mL/min)	2.965	0.88	0.24
7.	Column temperature (25˚C)	3.047	0.95	0.24
8.	Column temperature (27 ˚C)	3.002	0.88	0.19
9.	Column temperature (23˚C)	2.974	0.91	0.21

Table 7: System suitability Parameter

Resolution	6.95222
Column efficiency(No of theoretical Plate)	5584
Precision of replicate injection(Injection repeatability)(% RSD)	0.94
Asymmetry factor	0.95
Capacity Factor	1.05
HETP	4.47×10^{-3} cm

Table 8: Analysis result of Tablet dosage form

Brand Name	Formulation	Labeled amount (mg)	Amount found (mg)	Assay % ± SD
Banzel	Tablet	200	198.5	99.25 ± 0.29

Table 9: Summary of validation parameter

Linearity range	**10-80 µg/mL**
Regression	0.999
LOD	0.886976
LOQ	2.6878064
Intraday precision (% RSD)	0.04-0.73
Interday precision (% RSD)	0.08-1.48
Accuracy	97.15-100.6
Specificity	Specific

Table 10: Results of stressed degradation study

Stress Condition/duration	% Degradation
Acidic/ 1 N HCl, 80° C, 3 h	40.0
Alkaline / 1N NaOH, 80° C, 3 h	88.0
Oxidative/ 3%v/v H_2O_2	0.21
Photolysis/ sun light, 48 h	0.8
Thermal 100° C/ 12 h	0.20

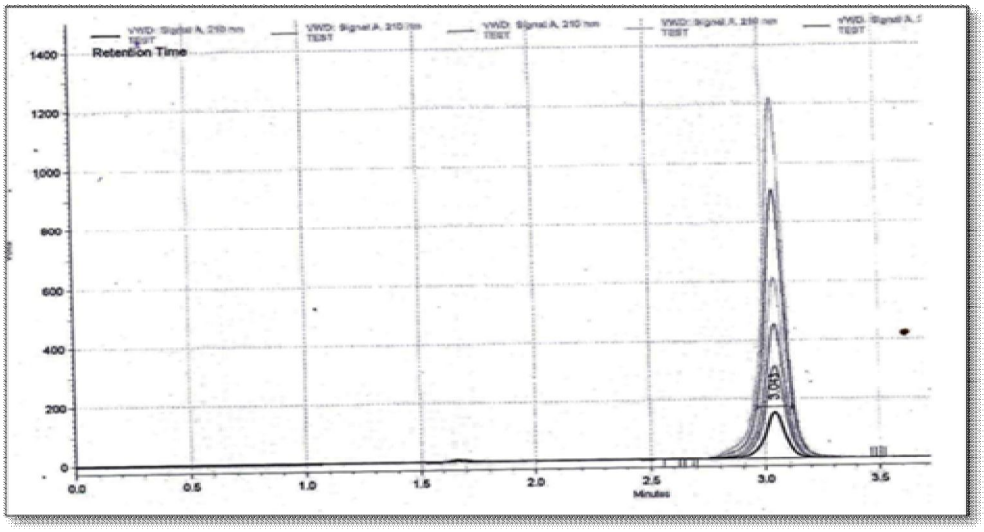

Figure 3: Chromatogram of Rufinamide calibration

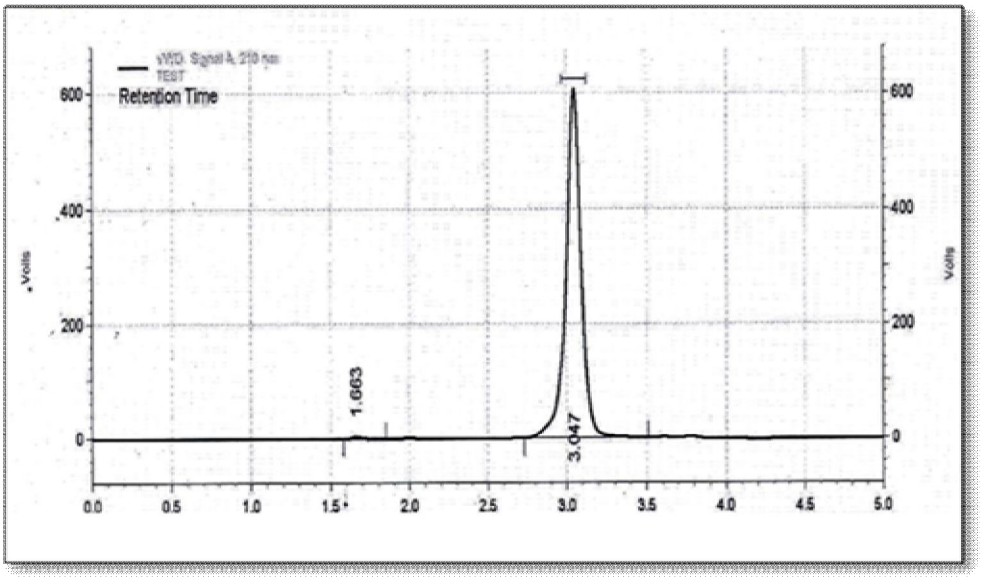

Figure 4: Chromatogram of Rufinamide Formulation

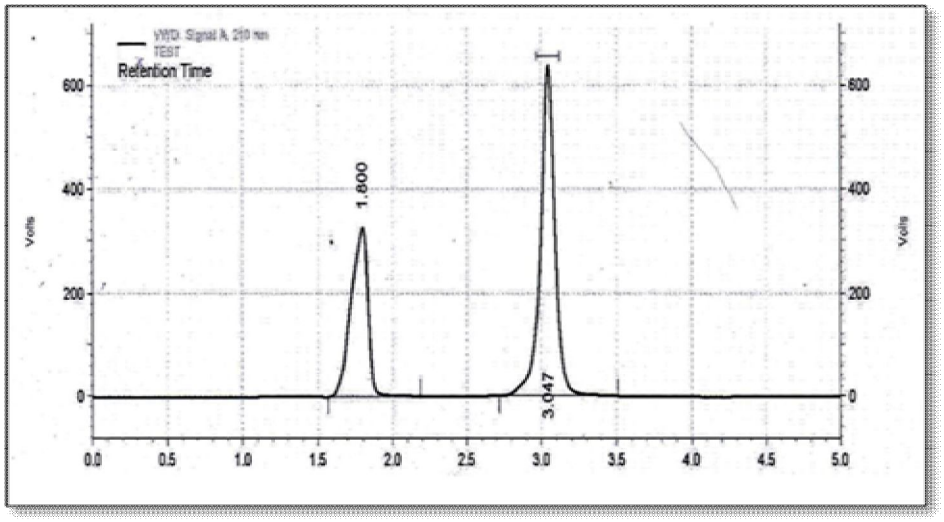

Figure 5: Chromatogram of Acid induced degradation

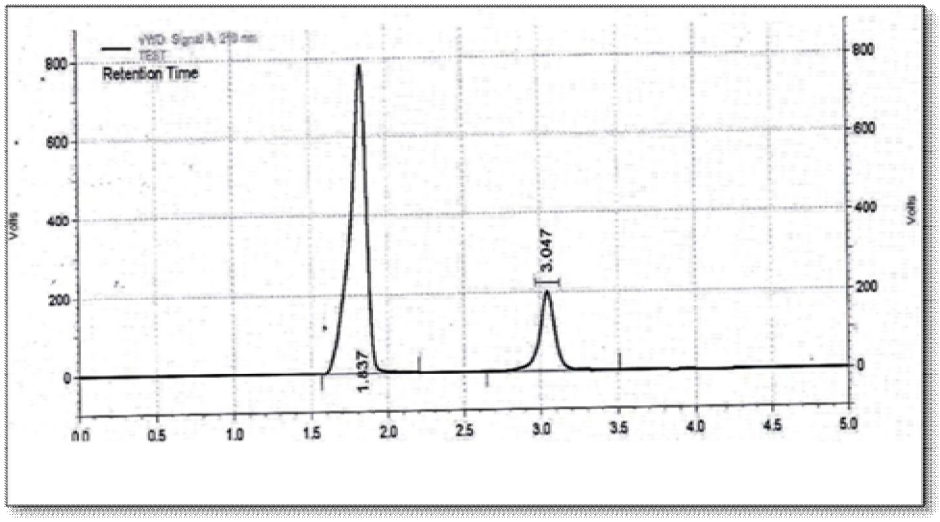

Figure 6: Chromatogram of Base induced degradation

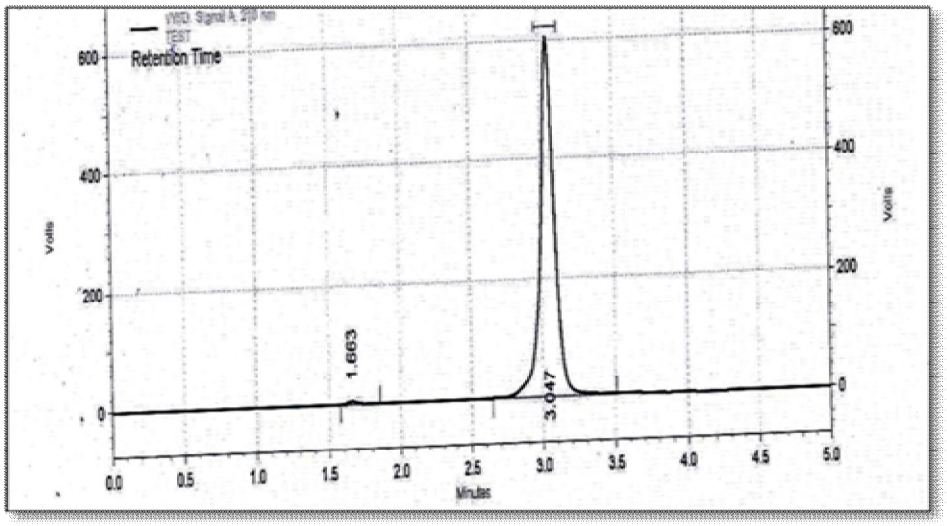

Figure 7: Chromatogram of thermal degradation

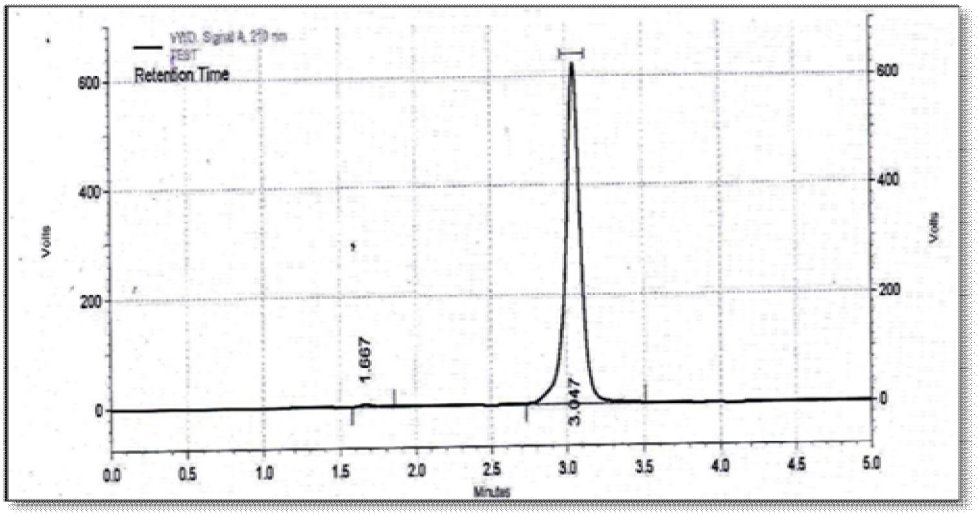

Figure 8: Chromatogram of oxidation of Rufinamide

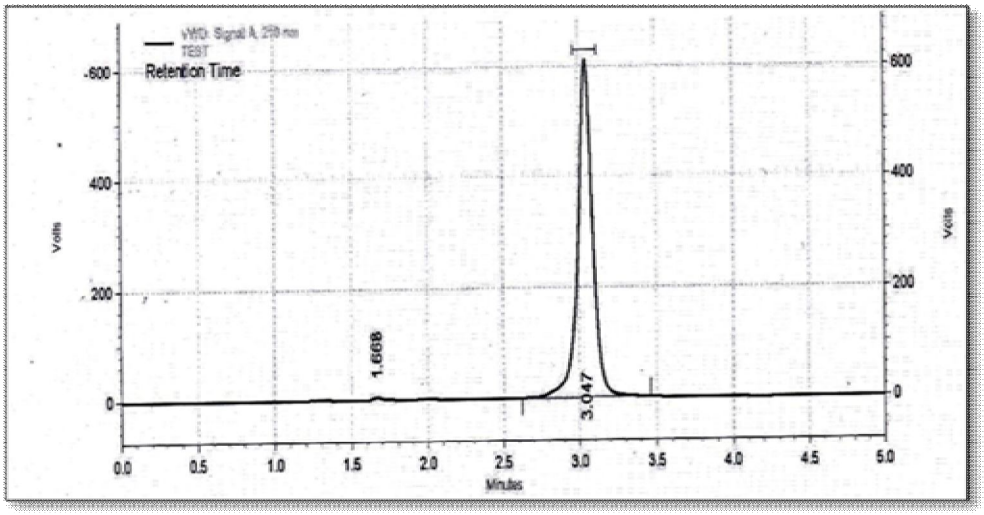

Figure 9 Chromatogram of photochemical degradation

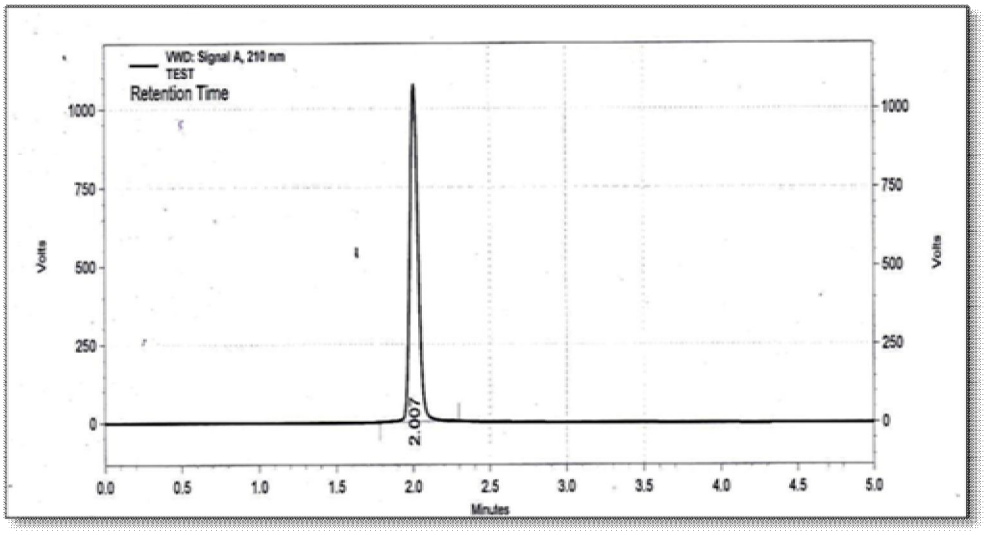

Figure 10: Chromatogram of degradation product of Rufinamide

CONCLUSION

Rufinamide is less stable in acidic and basic condition as compared to oxidation, photolysis and thermal degradation. All the peaks are resolved well from the standard rufinamide. This method is accurate, specific, economic and stability indicating method. It follows the validation parameters mentioned in ICH guideline. So this method can be used for routine analysis of pharmaceutical dosage form.

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