International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

"Optimization of UPLC Method for Quantitative Analysis of Rufinamide in Pharmaceutical Formulations Using Methanol-Water (50:50) Mobile Phase"

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Date of Submission: 20-06-2024 Date of Acceptance: 30-06-2024

ABSTRACT:
Accurate quantification of pharmaceutical compounds, such as rufinamide, is essential for ensuring the safety and efficacy of drug formulations. This study presents the development of an advanced Ultra-Performance Liquid Chromatography (UPLC) method for the precise estimation of rufinamide in pharmaceutical formulations. Rufinamide, an antiepileptic drug belonging to the triazole derivative class, poses challenges in quantification due to its unique chemical structure and limited solubility.The UPLC method development involved systematic optimization of chromatographic conditions, solvent selection, wavelength, and system suitability tests. Utilizing a Waters Cortex C18 column, an isocratic mobile phase composition of methanol and water (50:50 v/v) at a flow rate of 1.0 ml/min was identified for optimal separation. Detection was performed at 212 nm using a UV detector.The developed method exhibited excellent specificity, as confirmed by peak purity analysis. Linearity was established over a concentration range of 1.0–15.0 µg/ml, with a correlation coefficient (R^2) meeting the International Conference on Harmonisation (ICH) guidelines.Validation studies were conducted to assess the method's accuracy, precision, and robustness, which were found to be within acceptable limits. Filtration studies confirmed the compatibility of the chosen filter with the sample, while solution stability tests demonstrated the reliability of both standard and test solutions over a 24-hour period. Furthermore, the method exhibited excellent recovery (97.5–102.5%) and repeatability (%RSD < 2) for rufinamide.Validation of the developed method was performed according to ICH guidelines, covering specificity, linearity, accuracy, precision, and robustness. The proposed UPLC method offers a rapid, sensitive, and reliable approach for the quantification of rufinamide in pharmaceutical formulations, thereby facilitating quality control and assurance in drug manufacturing processes. This research contributes to the advancement of analytical techniques for pharmaceutical analysis, particularly in the field of antiepileptic drug development and quality assurance.

Keywords: Ultra-performance liquid chromatography (UPLC), method development, rufinamide, pharmaceutical analysis, chromatographic conditions, methanol-water ratio, isocratic separation, specificity, linearity, validation, quality control

I. INTRODUCTION

The antiepileptic medication on the market today is structurally unrelated to rufinamide, a triazole derivative. It dissolves almost completely in water, only very slightly in ethanol and acetonitrile, and somewhat in tetrahydrofuran and methanol. [1-2]

Fig: Chemical Structure of Rufinamide

A review of the literature showed that there are several different approaches that have been documented for estimating rufinamide in pharmaceutical formulations. This study focuses on the advancement of the HPLC technique. [2,3] Rufinamide in tablet dosage forms can be quantified using a straightforward, quick, and sensitive mobile phase. The devised method can

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

then be validated in accordance with ICH guidelines. Sonication of the sample at room temperature combined with sonication of a tiny amount of powder sample at room temperature was one of the key components and innovations of the suggested approach.

II. MATERIALS AND PROCEDURES

Materials

A gift sample of the medication rufinamide was received.

Chromatographic conditions and apparatus:

The HPLC apparatus (Agilent Model) consisted of a gradient quaternary pump and a multiple-wavelength PDA detector. Chromatographic separation was performed isocratically using a Phenomenex ODS-3 C-18 column (250mm x 4.6mm x i.d.). With a 2.7 µm mobile phase composition of a mixture of methanol (0.05% TFAA) and water (50:50) at a flow rate of 1.0 ml/min, a sample injection of 20 µl was injected. The eluent was monitored with a PDA detector set at 212 nm. The diluent was a mixture of methanol and water (50:50).

The mobile phase's preparation:

The ph of the mobile phase was adjusted to 0.01% TFAA and is composed of methanol and water in a 50:50 ratio. After passing the fluid through a 0.45-µ membrane filter made of nylon syringe, it was subjected to 15 minutes of intermittent shaking and sonication. This solution served as a movable phase for additional investigation.

Choosing a wavelength for analysis

Choice of Solvents

The solvent chosen to dissolve rufinamide was methanol.

Getting the standard solutions ready for UV scans

To make the stock solution, precisely weigh 25 mg of rufinamide, transfer it into a 50-ml volumetric flask, add 35 ml of methanol, sonicate to thoroughly dissolve the standard, and then dilute the mixture with methanol (500 PPM).

Solution for a UV scan:

After pipetting off 1.0 ml of the stock solution, dilute it with 25 ml of methanol. Twenty parts per million

Choosing a wavelength for analysis

From 400 nm to 200 nm, methanol was used as a blank and a standard solution of rufinamide (20 PPM). For this medication, absorption maxima were found. In the results, rufinamide's highest absorbance was measured at 212 nm.

UPLC's Method Development

Making a standard stock solution in advance of chromatographic development

25 mg of rufinamide were dissolved in a 50-ml volumetric flask that had been cleaned and dried. A total of 35 ml of methanol was then added to the flask to completely dissolve the rufinamide and bring the volume up to the required level (500 PPM).

Further diluted 2 ml of stock solution to 10 ml with mobile phase (100 PPM). It was prepared in the mobile phase of each trial and injected into the development trials. 212 nm was chosen as the analytical wavelength for the investigation based on the spectrophotometric analysis's highest absorption. This was done in order to build the UPLC method.

The conditions of chromatography

Standard solution:	Rufinamide 100 PPM
Detector:	U.V. Detector
Column:	Waters Cortex C18
Column Dimension:	(150 mm X 4.6 mm i.d.) 2.7µm
Column Oven temperature:	35°C
Injection Volume:	20 µl
Wavelength:	212 nm
Mobile phase:	Methanol: water (70:30)
Flow Rate:	1.0 ml/min

The UPLC Method's Validation

The proposed approach for the Vortioxetine estimate was confirmed in accordance with the ICH criteria for the following parameters:

FILTRATION STUDY:

The analytical process of the filtration study verifies that the filter is compatible with the

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

sample, that it does not clog the filter, and that no foreign materials are deposited on the filter bed. This investigation used a physical lab mixture, or rufinamide test sample. With both filtered and unfiltered test solutions, the filtration investigation was conducted. During the filtering process, 5 ml of the aliquot sample was discarded, and 0.45 µm PVDF and 0.45 µm Nylon syringe filters were utilized.

ANALYTICAL SOLUTION STABILITY

Stability analysis was carried out on both the test sample and the standard solution. The stability research was carried out in a typical laboratory setting. After 12 and 24 hours, the solution was evaluated under standard laboratory lighting conditions. By comparing the test solution results at each stability time point to the starting results, a standard and test solution stability study was carried out.

SPECIFICITY:

The capacity to clearly identify the analyte in the presence of potentially anticipated components is known as specificity. The next step is to prepare and inject the solution to demonstrate the method's specificity. (Peak purity for test sample and standard solution checked)
Blank (mobile phase as a diluent)
Placebo

Standard solution for rufinamide
Sample solution for tablet tests
Excipients, or additives, used in the analysis of marketing test samples are completely unknown. Thus, a placebo was created in a lab setting using the following formula:

Sr. No.	Ingredients	Role	Qty (mg)
1	Lactose	Filler	80
2	Starch	Binder	5
3	Magnesium stearate	Lubricant	5
4	Talc	Glidant	5
5	Crospovidone	Disintegrants	5
Total			100 mg

Overall, 10 grams of placebo were made:

Preparing the placebo sample solution:

Measured and transferred 25 mg of placebo material—which is equal to 25 mg of rufinamide—to a 50-ml volumetric flask that had been cleaned and dried. I added 35 ml of methanol and then shook intermittently while sonicating for five minutes. After ten minutes, let the mixture cool to ambient temperature and add methanol to get the volume up to the desired level. Using an appropriate 0.45 µ syringe filter, filter the solution, discarding the first 3–5 millilitres of filtrate. Chromatograms were acquired after further diluting 0.5 ml of the filtered stock solution to 25 ml with the mobile phase and injecting the resulting solution.

Range and linearity
Making the linearity solution

The capacity of an analytical method to produce test findings that are exactly proportionate to the concentration (amount) of analyte in the sample, within a specified range, is known as linearity.
Five different linearity levels, ranging from 10% to 150% of the working concentration, were tested.

Linearity of the stock solution for rufinamide: 25 milligrams of rufinamide were weighed and then dissolved in 25 ml of methanol. Add more methanol (50 PPM) to the 2.5 ml to make 50 ml.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

The following linearity levels were prepared:

Sr. No.	Level (%)	Mlof stock solution	Diluted to with mobile phase (ml)	Rufinamide Concentration (µg/ml)
1	10%	0.20	10	1.00
2	50%	1.00	10	5.00
3	100%	2.00	10	10.00
4	125%	2.50	10	12.50
5	150%	3.00	10	15.00

Determination

Every level was injected three times, and the mean area was determined. The calibration curve was visually represented as mean area on the y-axis versus analyte concentration in µg/ml on the X-axis, as shown by the results.

Standards of acceptance

Correlation Coefficient: NLT 0.98
Intercept: To be report
Slope: To be report

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

Detection limit:

The lowest concentration of analyte in a sample that can be identified but may not always be quantified as an exact number is known as the detection limit of a particular analytical technique.

Quantitation limit:

The lowest concentration of analyte in a sample that can be quantitatively identified with appropriate precision and accuracy is known as the quantitation limit of a particular analytical process. The method was used to determine LOD and LOQ in accordance with ICH Q2R1 recommendations. Utilizing the following formula, the LOD and LOQ were ascertained based on the calibration curve, which was used to calculate the residual standard deviation of a regression line:

$LOD = 3.3\ \sigma / S$
$LOQ = 10\ \sigma / S$
Where,
Σ represents the residual standard deviation of a regression line.
S = slope of the regression line.

Accuracy (% Recovery)

The degree of agreement between the value found and the value that is recognized as either a conventional true value or an acceptable reference value is expressed by the analytical procedure's accuracy. Between fifty percent and one hundred fifty percent of working concentration will be used for accuracy. Three copies of each accuracy level's solution were made. % recovery for each sample, mean recovery for every level, and total recovery were computed. Additionally, % RSD for every level and % RSD for the total recovery were computed.

Accuracy levels details:

Consult Each sample is listed in the table below:

Level (%)	API (mg)	Placebo	Diluted to (ml)	Volume taken	Diluted to (ml)	Conc (µg/ml)
50	12.6	25.6	50	0.5	25	5.04
	12.5	25.2	50	0.5	25	5.00
	12.7	24.7	50	0.5	25	5.08
100	25.1	25.1	50	0.5	25	10.04
	25.3	24.9	50	0.5	25	10.12
	25.2	25.6	50	0.5	25	10.08
150	37.8	25.2	50	0.5	25	15.12

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

	37.5	24.9	50	0.5	25	15.00
	37.6	25.8	50	0.5	25	15.04

How to prepare the accuracy sample solution:

Take a clean and dried 9-volumetric flask of 50 ml. Weighed approximately 25 mg of placebo and transferred it into each 50-ml volumetric flask. Wighed Rufinamide API as per accuracy level and transferred in the same 50-ml volumetric flask. Add 35 ml of methanol and sonicate it for 10 minutes with intermittent shaking. Allowed to cool the solution at room temperature and made the volume up to the mark with methanol. Filter the solution through a suitable 0.45 µ Nylon filter, discarding 5 ml of filtrate. Further dilute 0.5 ml of filtrate to 25 ml with the mobile phase.

Standards of acceptance

1. % recovery for each sample and the mean recovery and overall recovery should be in the range of 98–102%.
2. The relative standard deviation should not be more than 2.0%.

PRECISION

The degree of agreement between several measurements taken from numerous samplings of the same homogenous test conducted under the specified conditions is expressed as the precision of an analytical method. Repeatability and intermediate precision are the two categories of precision. It is carried out on a test sample of tablets.

Repeatability:

Sample solution preparation (six samples are made): The powder material equivalent to 25 mg of rufinamide was weighed out of the physical lab combination and then transferred to a 50-ml volumetric flask that had been cleaned and dried. I added 35 millilitres of methanol and then sonicated with sporadic shaking for ten minutes. Allow the solution to cool to ambient temperature after ten minutes, then add methanol to bring the volume up to the desired level. 3-5 ml of the initial filtrate were discarded after filtering the mixture using an appropriate 0.45 µ syringe filter. Using the mobile phase, further dilute 0.5 ml of the filtered stock solution to 25 ml. Chromatograms were recorded after injecting the resulting solution containing 10 mcg of rufinamide.

Six samples prepared.

The following are the sample details for precision (repeatability):

Sample	Powder WT (mg)	Diluted to (ml)	Volume taken	Diluted to (ml)
Sample 1	50.6	50	0.5	25
Sample 2	49.8	50	0.5	25
Sample 3	50.3	50	0.5	25
Sample 4	49.9	50	0.5	25
Sample 5	50.7	50	0.5	25
Sample 6	50.4	50	0.5	25

Standards of acceptance

% Assay: 90-110% for each sample and mean assay value

% RSD for % assay value of 6 samples: NMT 2%

In between precision levels

In order to verify the reproducibility of the results, analysis is carried out on a different day. Six samples were prepared using the same methodology as the Repeatability parameter.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

Intermediate Precision Sample details are as follows:

Sample	Powder wt. (mg)	Diluted to (ml)	Volume taken	Diluted to (ml)
Sample 1	49.7	50	0.5	25
Sample 2	50.6	50	0.5	25
Sample 3	50.3	50	0.5	25
Sample 4	49.9	50	0.5	25
Sample 5	50.4	50	0.5	25
Sample 6	50.1	50	0.5	25

Standards of acceptance
% Assay: 90-110% for each sample and mean assay value
% RSD for % assay of 6 samples of Intermediate precision: NMT 2
% RSD for Total 12 samples: NMT 2% for test results (6 of Repeatability and 6 of Intermediate precision)

ROBUSTNESS
An analytical procedure's resilience to tiny, intentional changes in method parameters is measured by its robustness, which also indicates how reliable it is under typical operating conditions.

Determination: As indicated below, injections of the Blank and Standard solutions were made under various chromatographic settings.
A). Variations of ±10% in the flow rate. (± 0.1 ml/min)
B). The temperature of the column oven has changed. (± 2ºc)
C) Wavelength variation (± 3 nm)

III. RESULT AND DISCUSSION

The preliminary identification and characterisation of the drug
The appearance, color, and scent
The drug's color, smell, and look

Sr. No	Name	Colour, odour and appearance of drug
1	Rufinamide	White, odourless and Amorphous powder

Solubility study
Solubility study of Rufinamide

Sr. No.	Name of Solvent	Observation	Conclusion	Summary
1	Water	Drug Particles seen after sonication	Drug was not found soluble in water.	Methanol used as a diluent for preparing stock solution.
2	Methanol	No Drug Particles seen after sonication	Drug was found soluble in methanol.	

Choice of solvent
Rufinamide was chosen to be dissolved in methanol.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

Selection of analytical wavelength

1) **Blank methanol:**

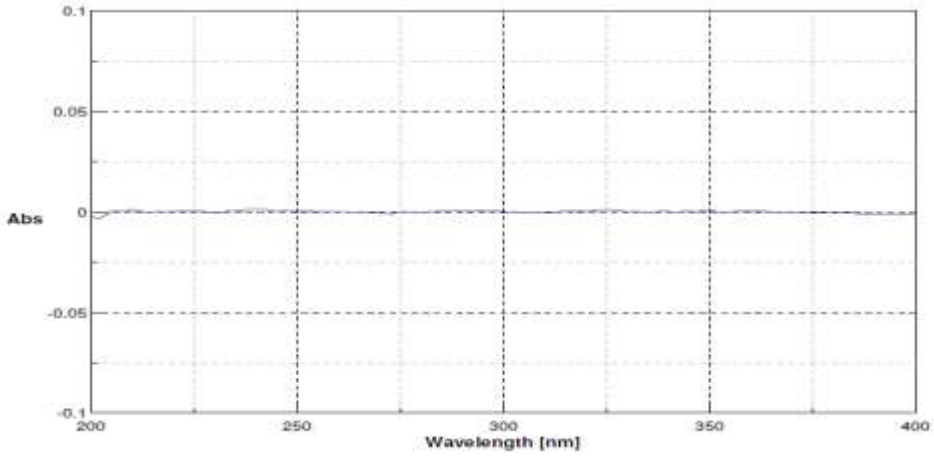


Fig. No. 1 UV spectrum of Methanol as a blank

2) **Rufinamide STD solution: (20 PPM)**

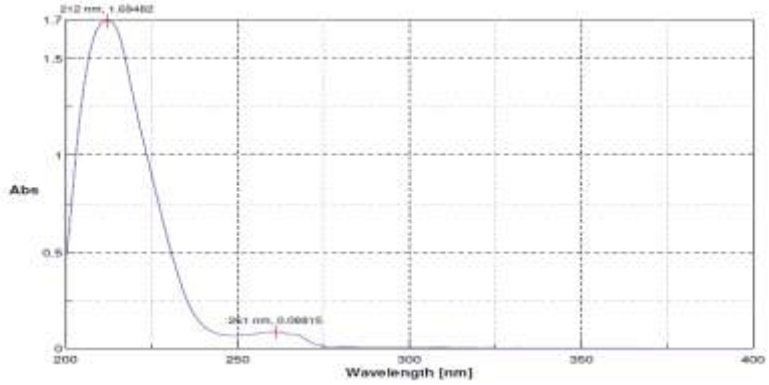


Fig. No. 2 UV spectrum of Rufinamide

Observation: The 400–200 nm range was scanned using the usual solution. The drug's maximum absorption wavelength was found. The absorbance of rufinamide peaked at 212 nm. You may see it in Figure No. 2. As a result, 212 nm is thought to be suitable analytical wavelength for additional research.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

Method Development by UPLC
Optimization of UPLC method
Chromatogram:

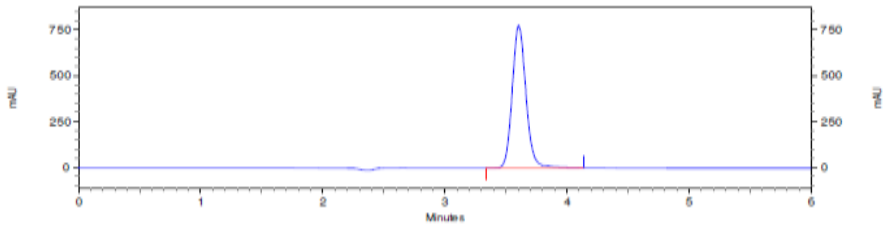


Fig. No.3 Typical chromatogram of Rufinamide

ObservationThe eluted rufinamide showed excellent chromatography. (Asymmetry: 1.38; plate theory: 5994)
Conclusion: After analyzing the data from trials one through six, it was determined that trial six's chromatographic conditions produced a better peak, a decent retention time, and a tailing factor; as a result, trial six's chromatographic conditions were utilized to validate the method.

Chromatographic Conditions Optimized

Parameter	**Description**
Mode	Isocratic
Column Name	Waters Cortex C18**,** 150 mm X 4.6mm ID, 2.7 µm
Detector	UV Detector
Injection Volume	20 µl
Wavelength	212 nm
Column Oven temp	35ºc
Mobile Phase	Methanol: 0.05% TFAA (50:50%V/V)
Flow Rate	1.0 ml/min
Run time	06 Minutes

System suitability test
Results for System Suitability Test of Rufinamide:

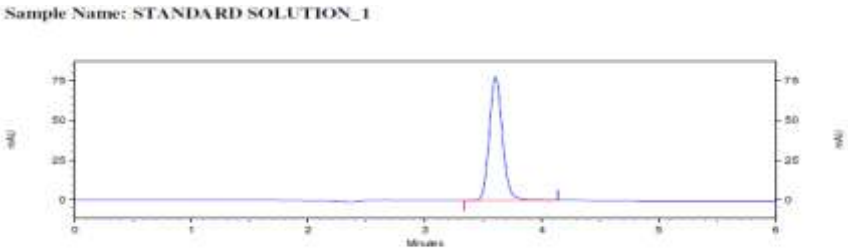
Sr. No.	**Standard solution**	**Area**	**Asymmetry**	**Theoretical plates**
1	Standard_1	9630256	1.34	6213
2	Standard_2	9649624	1.33	6235
3	Standard_3	9602560	1.33	6228
4	Standard_4	9650703	1.34	6234
5	Standard_5	9688231	1.33	6216
Mean		**9644275**	**1.33**	**6225**
STD Dev		**31379.66900**		
% RSD		**0.33**		

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

System Suitability Acceptance Criteria:

1. In standard chromatograms, the relative standard deviation of the analyte peak area shouldn't be higher than 2.0%.
2. In standard chromatograms, the theoretical plate count of the analyte peak should not be fewer than 2000.
3. In standard chromatograms, the analyte peaks' tailing factor (asymmetry) should be less than 2.0.

Data interpretation: As can be seen from the data given above, the procedure conforms with the requirements for system appropriateness. Therefore, it may be said that the intended analysis can be conducted using the chromatographic method.



VWD: Signal A, 212 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Rufinamide	3.61	9630256	1.34	6213
Totals		9630256		

Fig. No. 4Typical chromatogram of Standard solution 1 of system suitability solution.

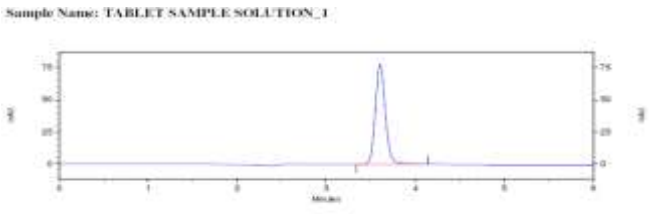
Physical lab combination assay

A) Physical lab mixture:

Average weight of tablet = 400 mg (Theoretical considered)

Assay results of Physical lab mixture

Sample	Area	% Assay	Mean Assay
Sample 1	9603587	98.40	98.87
Sample 2	9619253	99.34	



VWD: Signal A, 212 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Rufinamide	3.60	9603587	1.34	6250
Totals		9603587		

Fig. No. 5 Typical chromatogram of Physical lab mixture.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

Acceptance criteria:
1) The assay result percentage should be between 95 and 105%.
Data interpretation: Based on the aforementioned findings, it can be said that the sample can be utilized for validation and that the assay result is within the range for a physical lab combination.

VALIDATION OF RP-UPLCMETHOD
FILTRATION STUDY: An analytical procedure's filtration research examines the influence of extraneous components from the filter, deposition on the filter bed, and compatibility of the filter with the sample. Carried out on a tablet test sample.

Results of Filter study

Sample description	Area	% Absolute difference
Unfiltered	9642586	NA
0.45 µ PVDF filter	9538014	1.08
0.45 µ Nylon filter	9558109	0.88

Chromatograms:

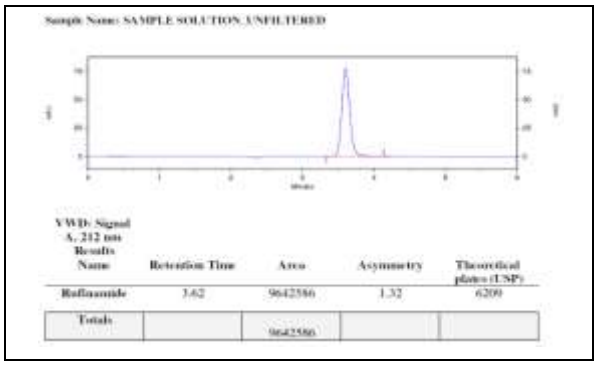


Fig. No. 6 Typical chromatogram of unfiltered sample.

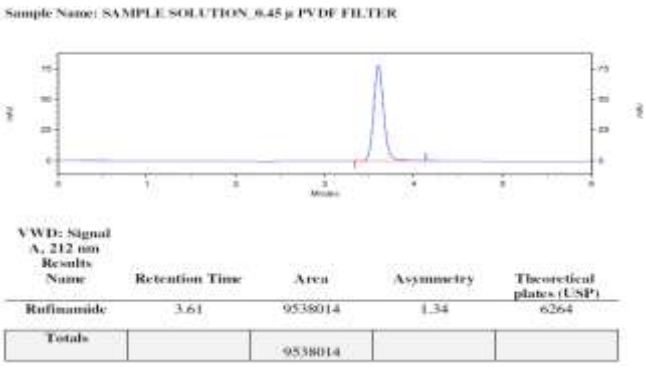


Fig. No. 7 Typical chromatogram of sample filtered through 0.45µ PVDF filter.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

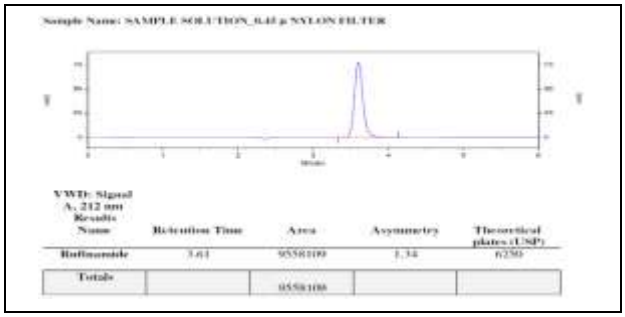


Fig. No. 8 Typical chromatogram of sample filtered through 0.45µ Nylon filter.

Acceptance criteria: % Absolute difference of filtered samples NMT 2.0 w.r.t. Unfiltered sample.
Data interpretation: Both filters PVDF and Nylon passes the criteria for filter study, hence both filters can be used. We used Nylon filter because it showed less absolute difference as compare to PVDF filter.

SOLUTION STABILITY: Stability study was conducted for Standard as well as Test Sample. Stability study was performed at normal laboratory conditions. The solution was stored at normal illuminated laboratory conditions and analysed at initial, after 12 hours and 24 hours.

Results of Solution stability.

Sample solution			**Standard solution**		
Time point	**Area**	**% Absolute difference**	**Time point**	**Area**	**% Absolute difference**
Initial	9630478	NA	**Initial**	9662047	NA
12 Hours	9567956	0.65	**12 Hours**	9606582	0.57
24 Hours	9530256	1.04	**24 Hours**	9580267	0.85

Chromatograms:

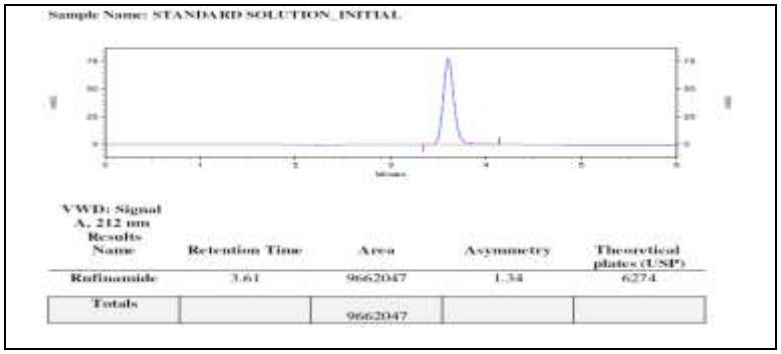
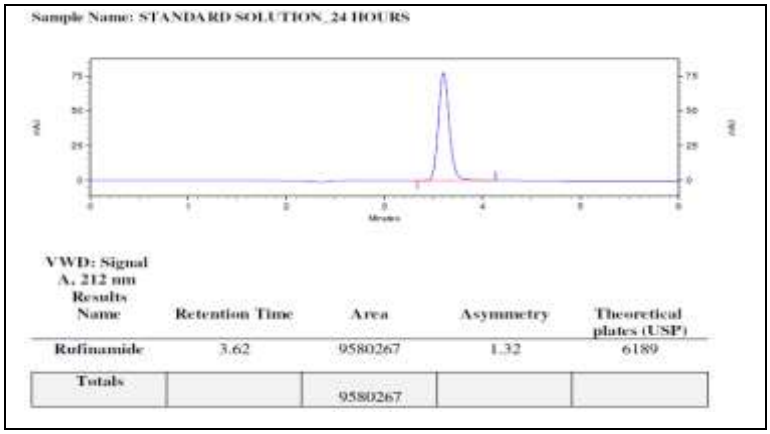


Fig. No. 9 Typical chromatogram of Standard solution Initial.

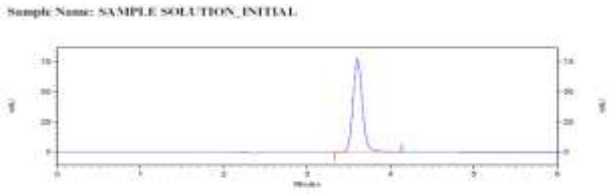
International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494



VWD: Signal A, 212 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Rufinamide	3.62	9580267	1.32	6189
Totals		9580267		

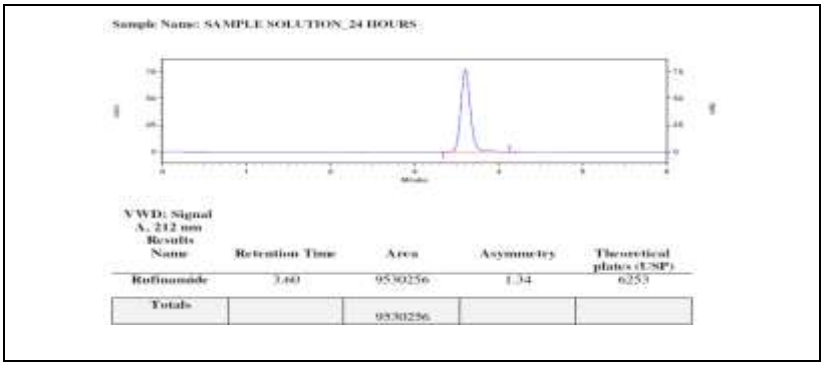
Fig. No. 10 Typical chromatogram of Standard solution After 24 Hrs.



VWD: Signal A, 212 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Rufinamide	3.61	9630478	1.35	6237
Totals		9630478		

Fig. No. 11 Typical chromatogram of Test solution Initial.



VWD: Signal A, 212 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Rufinamide	3.60	9530256	1.34	6253
Totals		9530256		

Fig. No. 12Typical chromatogram of Test solution After 24 Hrs.

Typical chromatogram of Test solution After 24 Hrs.

Standards of acceptance: NMT 2.0's absolute stability solution differs from the first solution in percentage terms.

Data interpretation: Testing and standard solutions were proven to be steady for a whole day. Therefore, you can use both solutions for up to 24 hours.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

SPECIFICITY: The capacity to clearly identify the analyte in the presence of potentially anticipated components is known as specificity.

Blank, standard solution prepared and injected to check peak purity.

Results of Specificity.

Description	Observation
Blank	No interference at R.T. of Rufinamide due to blank
Placebo	No interference at R.T. of Rufinamide due to placebo
Standard solution	Peak purity was 0.984
Test Solution	Peak purity was 0.978

Chromatograms:

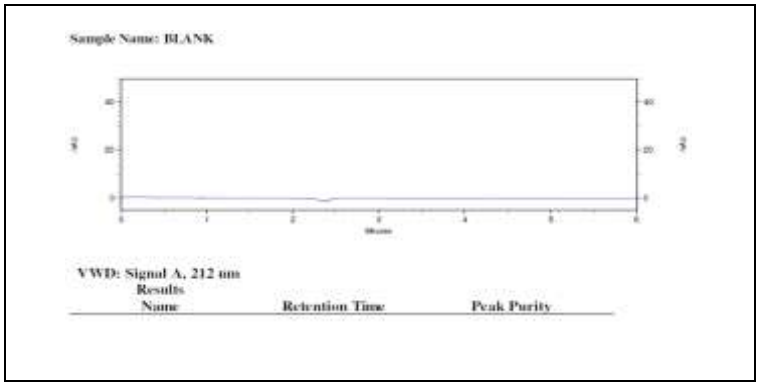


Fig. No. 13 Typical chromatogram of Blank solution.

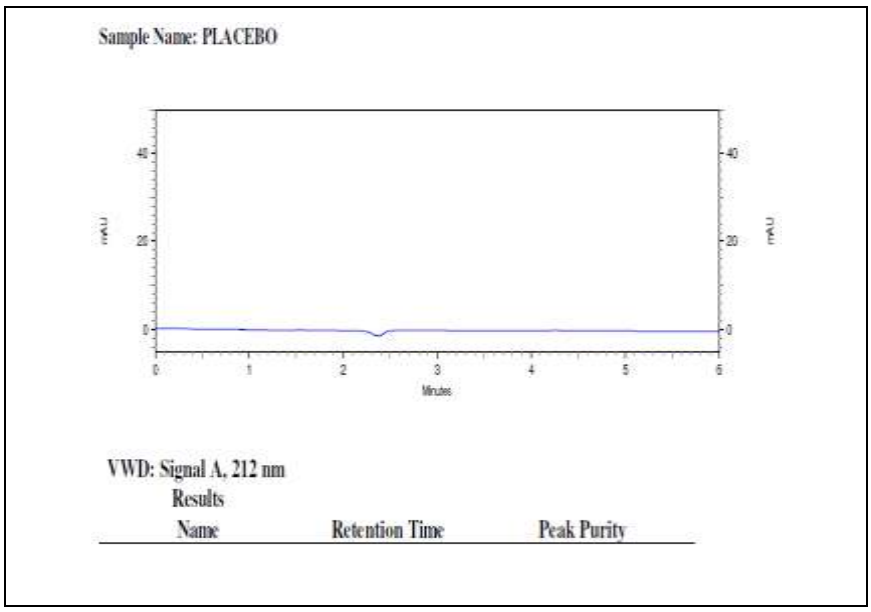


Fig. No. 14 Typical chromatogram of Placebo solution.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

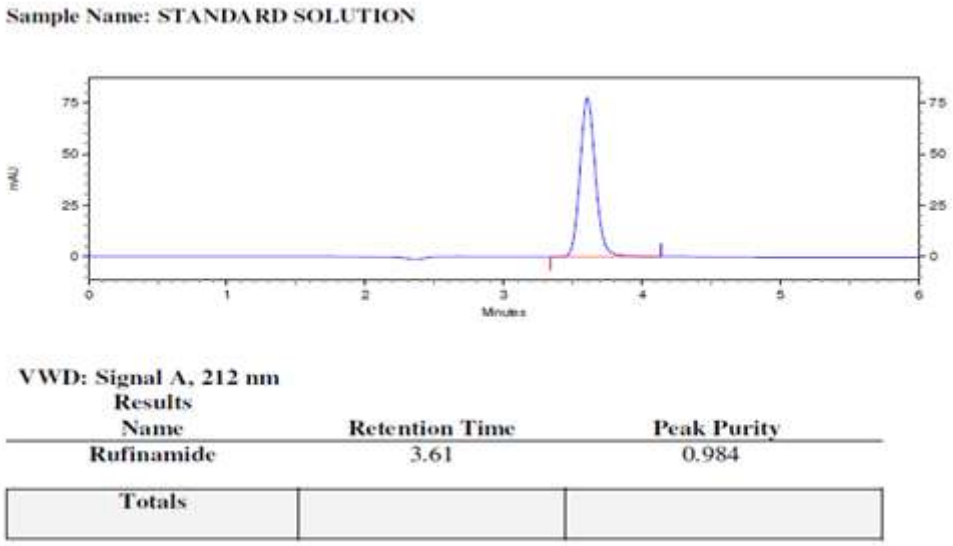


Fig. No. 15 Typical chromatogram of Peak purity of Standard solution.

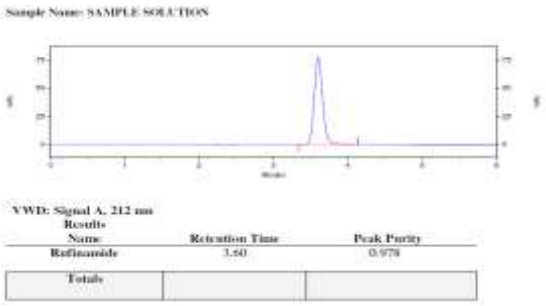


Fig. No. 16 Typical chromatogram of Peak purity of Test sample solution.

Standards of acceptance

Blank: There shouldn't be any disruption at Rufinamide's R.T.

Placebo: There shouldn't be any disruption at Rufinamide's R.T.

Standard and Test sample solution: Peak purity: NLT 0.95

Data interpretation: R.T. of rufinamide was not interfered with by blank or placebo. Both the Standard and test solutions' peaks of purity were substantially within acceptable bounds. As a result, the devised chromatographic technique met the specificity requirements.

Linearity and Range

The capacity of an analytical method to yield test findings that are proportionate to the analyte concentration in samples within a specified range is known as linearity.

Linearity Data for Rufinamide

Level	Conc (µg/ml)	Area	Mean	% RSD
10%	1.00	959605	961481	0.291
		964692		
		960145		
50%	5.00	4862575	4860877	0.304

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

		4845304		
		4874752		
100%	10.00	9655893	9655223	0.245
		9631250		
		9678527		
125%	12.50	11932567	11973905	0.314
		12005893		
		11983256		
150%	15.00	14325078	14313393	0.352
		14258210		
		14356891		

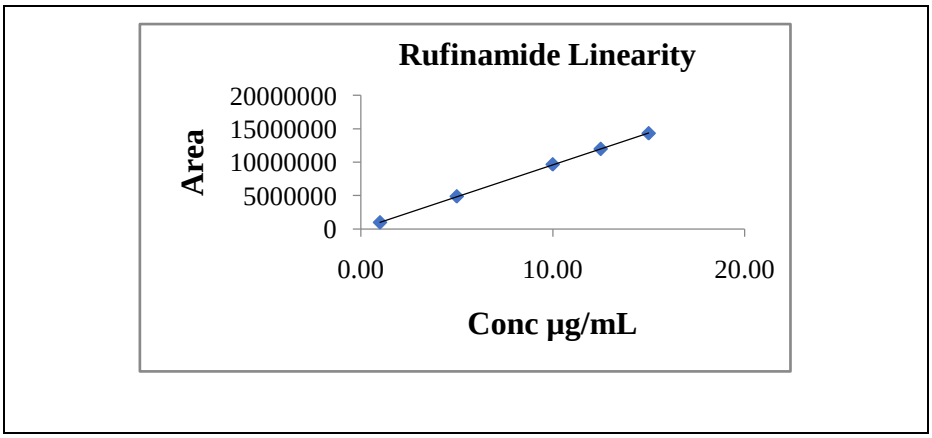


Fig. No. 17 Calibration curve of Rufinamide

Data of linearity of Rufinamide:

Sr no.	Parameter	Result value	Acceptance criteria
1	Beer's linearity range	1.0-15 µg/ml	NA
2	Correlation coefficient (R^2)	0.99996	NLT 0.98
3	Intercept	55664.47283	To be report
4	Slope	953713.9457	To be report
5	% RSD for area at each level	NA	NMT 2.0

The respective linear equation for Rufinamide was
Y = M X + C
Y = 953713.9457 x + 55664.47283
Where, x = concentration of Analyte in µg/ml
Y = is area of peak.
M = Slope
C= Intercept

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

Chromatograms:

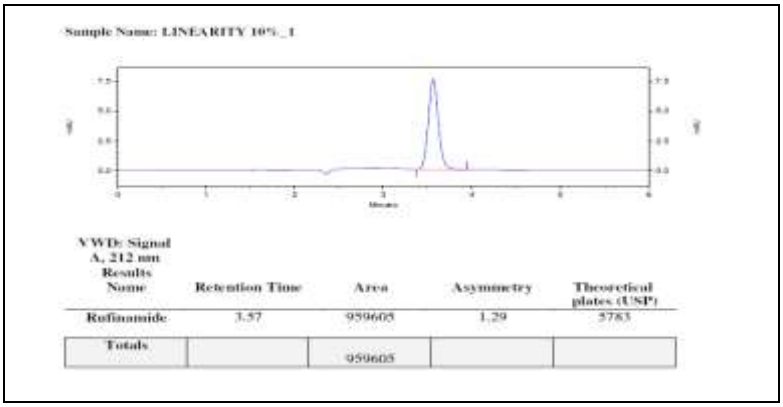


Fig. No. 18 Typical chromatogram of Linearity 10%.

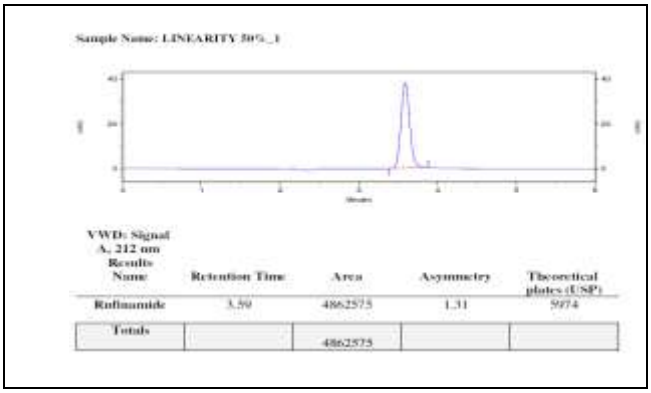


Fig. No. 19 Typical chromatogram of Linearity 50%.

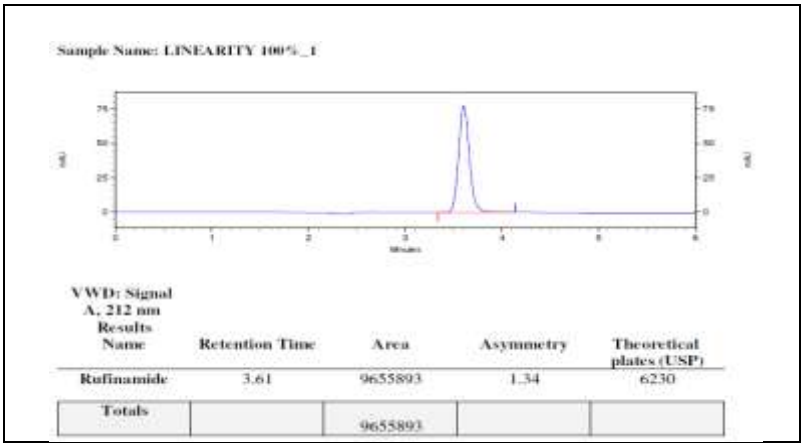


Fig. No. 20 Typical chromatogram of Linearity 100%.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

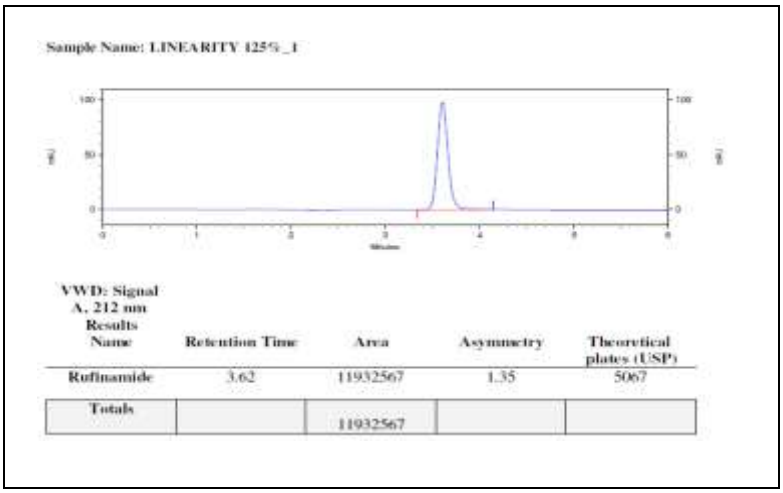


Fig. No. 21 Typical chromatogram of Linearity 125%.

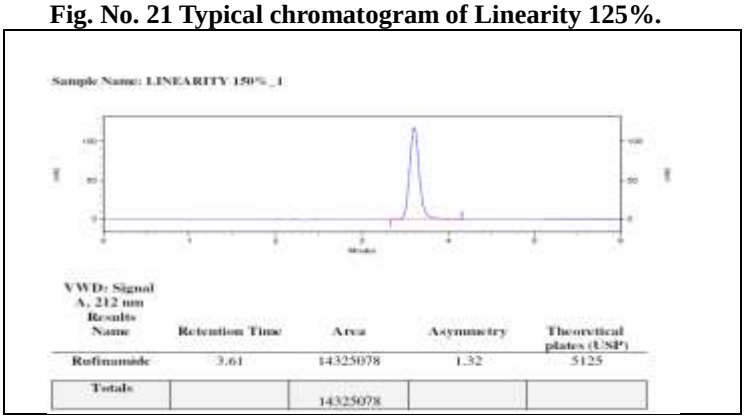


Fig. No. 22 Typical chromatogram of Linearity 150%.

Conclusion:

It was determined from the calibration curve that the rufinamide exhibits a linear response within the 1.0–15.0 µg/ml range. It was discovered that the regression value was well within the bounds.

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

Σ = 49611.687(Residual standard deviation of a regression line)
S = 953713.9457 (Slope)

Detection limit (LOD):

LOD = 3.3 σ / S
LOD = 3.3 x 49611.687/ 953713.9457
LOD = 0.17 µg/ml

Quantitation limit (LOQ):

LOQ = 10 σ / S
LOQ = 10 x 49611.687/ 953713.9457
LOQ = 0.52 µg/ml

ACCURACY (RECOVERY):

The degree to which test findings produced by an analytical method closely resemble the underlying value is known as the method's accuracy. Analyzed samples that have been subjected to known concentrations of analyte are used to assess an analytical method's accuracy.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

Result and statistical data of Accuracy of Rufinamide

Level (%)	Area	Recovered conc (µg/ml)	Added conc (µg/ml)	% Recovery	Mean Recovery	% RSD
50	4823568	5.00	5.04	99.21	**99.48**	**1.0219**
	4851250	5.03	5.00	100.60		
	4833068	5.01	5.08	98.62		
100	9658213	10.01	10.04	99.70	**99.01**	**0.6251**
	9643256	10.00	10.12	98.81		
	9575913	9.93	10.08	98.51		
150	14732561	15.28	15.12	101.06	**99.71**	**1.2511**
	14385935	14.92	15.00	99.47		
	14303558	14.83	15.04	98.60		

Overall Recovery: 99.40 %
% RSD for Overall Recovery: 0.922
Chromatograms:

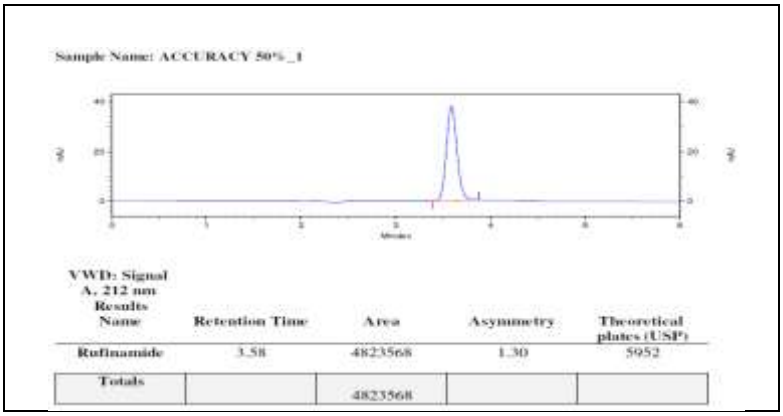


Fig. No. 23 Typical chromatogram of Accuracy 50%.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

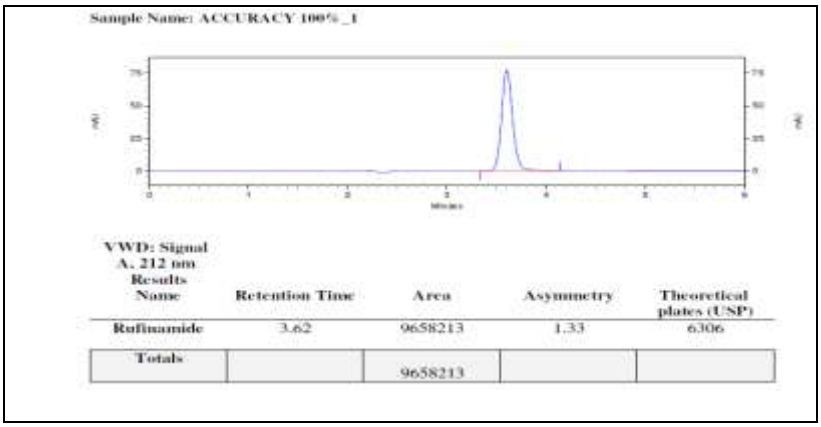


Fig. No. 24 Typical chromatogram of Accuracy 100%.

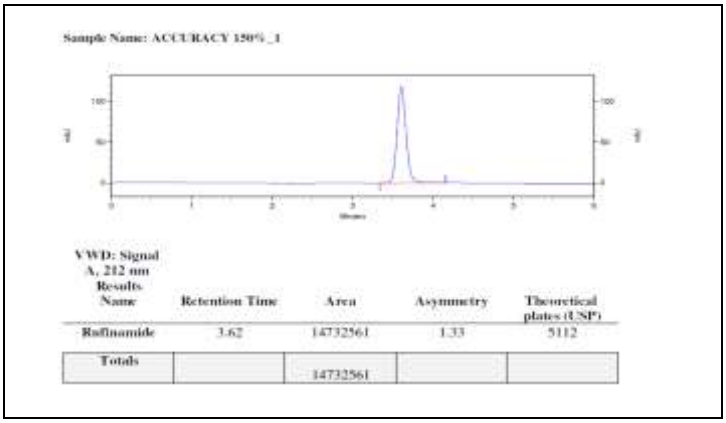


Fig. No. 25 Typical chromatogram of Accuracy 150%.

Standards of acceptance

Recoveries at each level and overall range from 98.0 to 102.0 percent.

RSD percentage for each stage and total recovery: NMT 2.0

Data interpretation: At all three levels, the analytical procedure's recovery was determined to be well within the acceptable limits. Analyte concentration changes do not impair recovery.

PRECISION

The degree of agreement between individual test findings obtained from repeatedly applying an analytical technique to multiple samplings of a homogenous sample is known as the precision of the method. Standard deviation or relative standard deviation are commonly used to express the precision of an analytical process. On the test sample, precision was applied.

Result of Intra- day and Inter- Day Precision for Rufinamide test sample assay:

	Sample	Test Sample (mg)	Area	% Assay
Repeatability	Sample 1	50.6	9506589	97.40
	Sample 2	49.8	9506258	98.96
	Sample 3	50.3	9400791	96.89
	Sample 4	49.9	9574560	99.48
	Sample 5	50.7	9614904	98.32

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

	Sample 6	50.4	9518903	97.92
	Mean			**98.16**
	STD DEV			**0.964996**
	% RSD			**0.983**
Intermediate precision (Inter-Day)	Sample 1	49.7	9480379	98.89
	Sample 2	50.6	9672154	99.10
	Sample 3	50.3	9542760	98.36
	Sample 4	49.9	9437684	98.05
	Sample 5	50.4	9524379	97.97
	Sample 6	50.1	9416034	97.44
	Mean			**98.30**
	STD DEV			**0.616877**
	% RSD			**0.628**
Repeatability Plus Inter-day	**Mean**			**98.232**
	STD DEV			**0.77563**
	% RSD			**0.790**

Chromatograms:

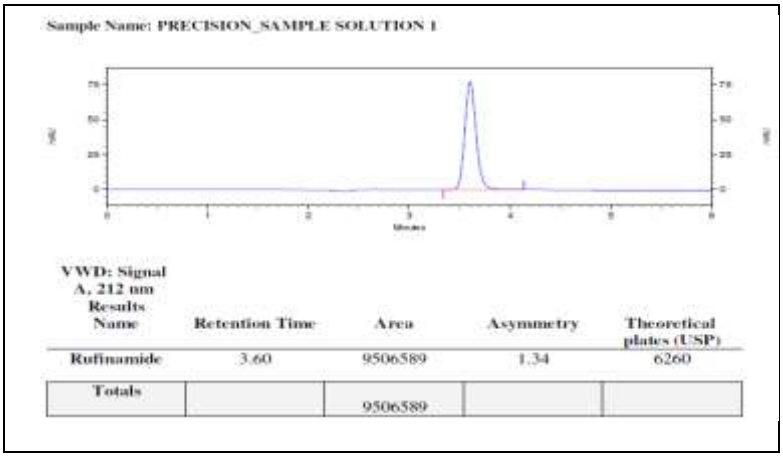


Fig. No. 26 Typical chromatogram of Repeatability precision (Sample 1).

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

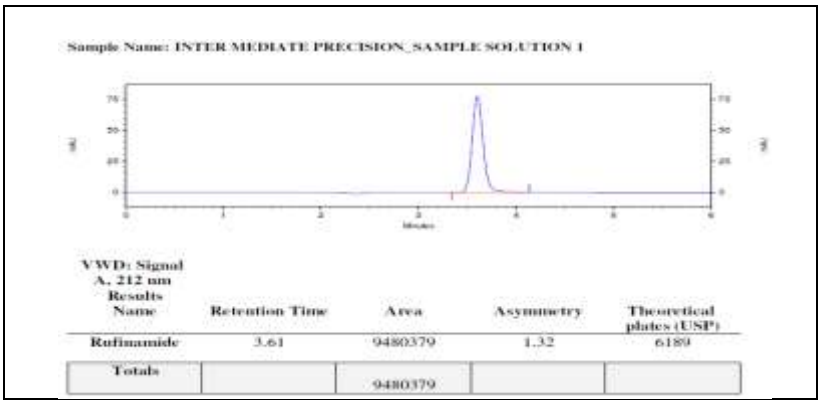


Fig. No. 27 Typical chromatogram of Inter-day precision (Sample 1).

Standards of acceptance:
% Assay: Each sample's assay value (individual sample), the mean assay value for precision (6 samples), the mean assay value for intermediate precision (6 samples), and the mean assay value for precision plus intermediate precision sample (12 samples) are indicated as follows: 90–110 percent
% RSD: The percentage RSD for six precision study samples, six intermediate precision study samples, and twelve precision plus intermediate precision samples is as follows: NMT Version 2.0
Data interpretation: Since the percentage RSD and assay were determined to be well within the acceptability limit, the procedure is exact (reproducible).

ROBUSTNESS:
An analytical technique's robustness gives an indication of its dependability under typical operating conditions by measuring its ability to withstand slight but intentional changes in method parameters.
Following changes made under Robustness:
Change in Wavelength
Change in flow rate
Change in column oven temperature

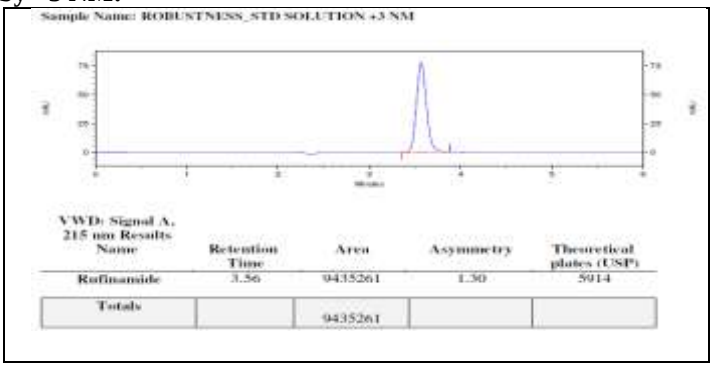
Result of Robustness study:

Change in Parameter	**R.T.**	**Standard area**	**Asymmetry**	**Theoretical plates**
Wavelength by +3 NM (215 NM)	3.56	9435261	1.30	5914
Wavelength by -3 NM (209 NM)	3.57	8745860	1.34	6117
Flow rate by +10% (1.1ml/min)	3.22	9268593	1.27	5473
Flow rate by -10% (0.9ml/min)	3.94	10186208	1.25	5160
Column oven temp by +2ºc (37 ºc)	3.59	9658213	1.35	6158
Column oven temp by -2ºc (33 ºc)	3.60	9586047	1.36	6291

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

Chromatograms:

Change in Wavelength by +3 NM:

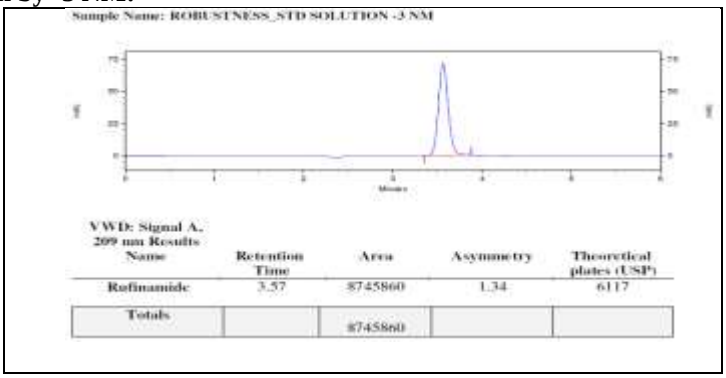


VWD: Signal A, 215 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Rufinamide	3.56	9435261	1.30	5914
Totals		9435261		

Fig. No. 28 Typical chromatogram of Standard +3 NM.

Change in Wavelength by -3 NM:

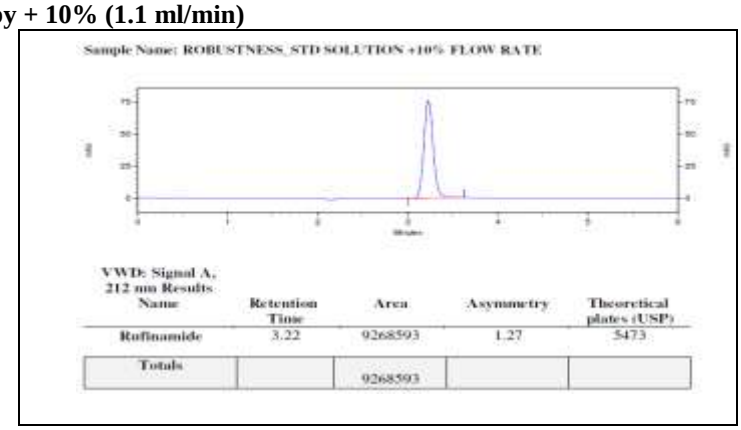


VWD: Signal A, 209 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Rufinamide	3.57	8745860	1.34	6117
Totals		8745860		

Fig. No. 29 Typical chromatogram of Standard -3 NM.

Change in Flow rate by + 10% (1.1 ml/min)



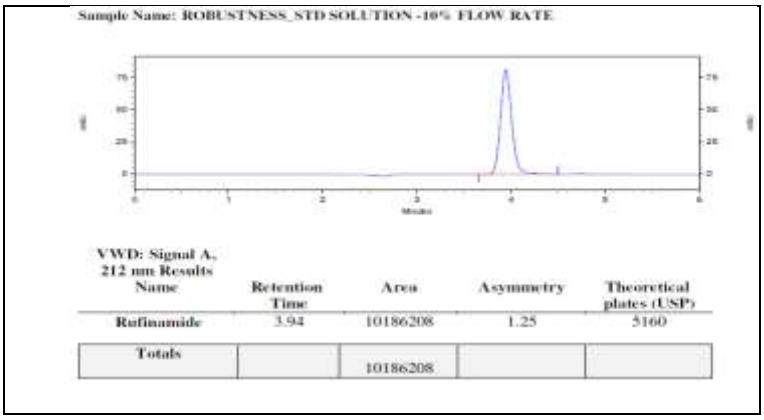
VWD: Signal A, 212 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Rufinamide	3.22	9268593	1.27	5473
Totals		9268593		

Fig. No. 30 Typical chromatogram of Standard +10 F.R.%.

Change in Flow rate by - 10% (0.9 ml/min)

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 2481-2504 www.ijprajournal.com ISSN: 2456-4494

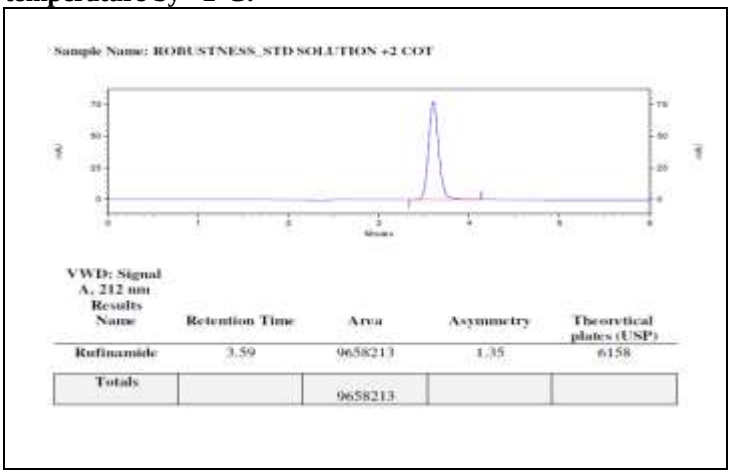


VWD: Signal A, 212 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Rufinamide	3.94	10186208	1.25	5160
Totals		10186208		

Fig. No. 30 Typical chromatogram of Standard -10 F.R.%.

Change in Column Oven temperature by +2°C:

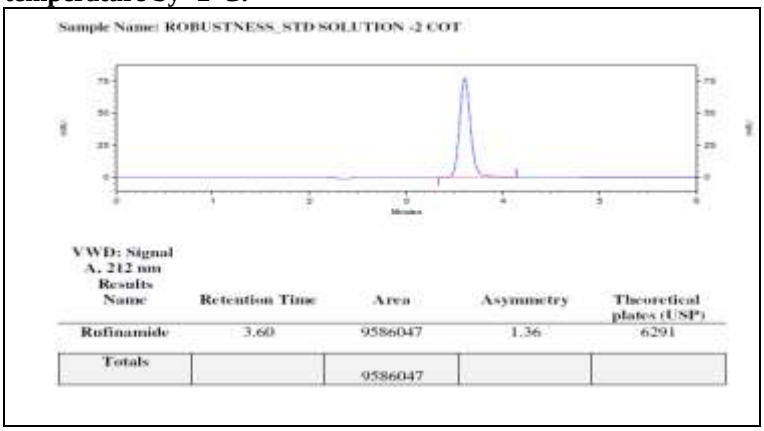


VWD: Signal A, 212 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Rufinamide	3.59	9658213	1.35	6158
Totals		9658213		

Fig. No. 31 Typical chromatogram of Standard +2°C C.O.T.

Change in Column Oven temperature by -2°C:



VWD: Signal A, 212 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Rufinamide	3.60	9586047	1.36	6291
Totals		9586047		

Fig. No. 32 Typical chromatogram of Standard -2ºc C.O.T.

Standards of acceptance

The acceptance requirements for chromatography (system appropriateness) shouldn't be broken.

Data interpretation: Based on the aforementioned findings, it was determined that the analytical method was reliable and the system suitability test result was found to be well within the limitations.

IV. CONCLUSION

"In conclusion, the developed UPLC method employing a methanol-water (50:50) mobile phase ratio demonstrated excellent performance in the analysis of rufinamide in pharmaceutical formulations. The method exhibited high specificity, sensitivity, and linearity over the tested concentration range. Validation results confirmed the reliability and accuracy of the developed method for routine quality control analysis. The isocratic separation approach facilitated rapid analysis without compromising the resolution of the target compound. Overall, the developed UPLC method offers a robust and efficient analytical tool for the quantification of rufinamide in pharmaceutical formulations, contributing to improved quality control and assurance in drug manufacturing processes."

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