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Analytical Method Development and Validation for Assay of Rufinamide Drug

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Abstract A simple, rapid, sensitive, cost effective and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the stability testing of rufinamide. The proposed RP-HPLC method was developed on phenome-nex Luna^R C-18 5µm ,250 mm × 4.6 mm id. Column (at ambient temperature) and mobile phase consisting of phosphate buffer: acetonitrile (60:40) was delivered at a flow rate 1.0ml/min. The analyte was detected by using UV detector at the wavelength of 293 nm. The method was found to be linear over the concentration range of 50-150 µgml^{-1} (r^2=0.999). 30. The retention time of rufinamide was 4.717 min.

Keywords: RP-HPLC, Rufinamide, API, Method Validation

1. INTRODUCTION

Stability testing forms an important part in the process of drug product development. Active pharmaceutical ingredient (API) is the important part of drug formulation and drug degrades with time so there is a need to develop methods which can detect degradation as well as degraded products (Chafez, L., 1971 and Sethi, P.D., 2001). The purpose of stability testing is to provide evidence on how the quality of drug substance varies with time under the influence of variety of environmental factors such as temperature, humidity and light which enables recommendation of storage conditions, retest periods and shelf life.

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1.1 Drug Profile (Rufinamide)

Description: (1-[(2, 6-difluorophenyl)methyl]triazole-4-carboxamide)

Rufinamide is an anti-epileptic is an structural analogue of MK801, carbamazepine and valproic acid recent analogue to gabapentin.

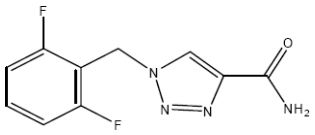


Figure: Rufinamide structure

Physiochemical properties Rufinamide

Parameter	Values
Molecular Weight	238.19
Physical state	White crystalline powder
Melting point	237 - 240^{0}C
Solubility in Water	Very low Soluble in water and soluble in THF and methanol
Stability	Stable under ordinary conditions.
Assay	98.5%
Residue on Ignition	0.15% max
Loss on Drying	9.5-12.5% max
Optical Rotation	-0.82^0
Heavy Metal	≥ 20 ppm
Category	Anti-epileptic Drug
State	Solid
Half Life	6-10 hours

1.2 Method Development

Number of methods are available to carryout stability indicating assay e.g. gas chromatography and nuclear magnetic resonance (NMR) (Chatwal, G., Anand, S.K., 2004 and Riley, M., Rosanke, T.W., 1996). High performance liquid chromatography (HPLC) is supposed to be the most efficient because it utilizes columns packed with very smaller particles and higher flow rate, which provides improved resolution, speed and sensitivity. Additional advantage of HPLC is that stability indicating assay of drugs can be carried out effectively in a short time (Boubakar, B.B., Etienne, R., et al., 2001; Hong, D., Shah, M., 2000 and Kar, A., 2005).

2. EXPERIMENTAL WORK

2.1 Materials

2.1.1 Instruments

The HPLC system consisted of HPLC 10AT-VP (Shimdadzu corporation ltd., Kyoto, Japan), manual injector port with 20 µL fixed loop (Rheodyme USA), UV detector SPD-20A (Shimadzu Corporation Ltd., Kyoto, Japan) and LC-20 AT (Prominenceseries) pumps were used. Separation was carried out on phenomenax C18, column (250 mm × 4.6 mm, 5 µm) Japan. Detector output was quantified on Spinchrom CFR chromatography software. Mettler-Toledo International Inc. Greifensee (Switzerland) microbalance was used for the purpose of weighing, and spinix vortex was used for the mixing. Samples were injected into HPLC system using Hamilton micro syringe.

2.2 Chemicals and Reagents

A gift sample of pure rufinamide was supplied from Varda Biotech pvt. Limited, Mumbai. Acetonitrile, water, methanol, potassium dihydrogen phosphate and triethylamine were procured from M/S Qualigens Fine Chemicals, Mumbai.

2.3 Methods

2.3.1 Selection of wavelength

10 ppm of drug solution was prepared by dissolving 100 mg of drug in small amount of methanol and volume was adjusted to 100 mL; after that 1 ml of above solution was diluted to 100 mL with methanol to get final concentration of 10 ppm. The solution was scanned in the U.V range of 200 nm to 400 nm (Green, J.M., 1996).

2.3.2 Preparation of Mobile Phase

For the analysis of rufinamide the aqueous system selected was phosphate buffer. Dissolved 6.08 Gm of potassium dihydrogen phosphate in sufficient water to produce 1000mL. pH was adjusted with glacial acetic acid using pH meter. After that buffer was mixed and sonicated with organic solvent i.e. acetonitrile in the ratio of 60 : 40 (Taylor and Francis, 2007).

2.3.3 Preparation of Calibration Curve

Stock solution (50 $\mu g mL^{-1}$) of rufinamide was prepared by dissolving in mobile phase. The stock solution of rufinamide was further diluted with mobile phase

to give the series of standard dilution for preparation of calibration curve (Taylor and Francis., 2007). The different concentration of sample solution (12.5, 25, 50, 62.5, 75, 100 μgml^{-1}) was injected in the concentration range of 50% - 150% of drug substanceand the Calibration Curve results are presented in Table 1 and Figure 1.

Table 1: Linearity data for Rufinamide

S.No.	Concentration/ μgml^{-1}	Inj-1/Area	Inj-2/Area	Inj-3 Area	Average area count/mV*sec
1	12.5	336	338	340	338
2	25	668	662	674	668
3	50	1368	1333	1340	1337
4	62.5	1680	1696	1703	1693
5	75	1998	2009	2008	2005
6	100	2675	2688	2650	2671
				Slope	26.71
				Intercept	4.71
				R^2	0.999

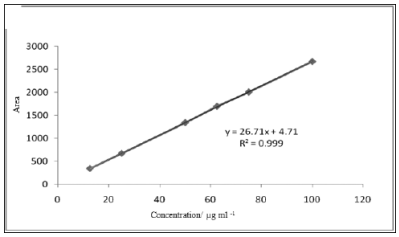


Figure 1. Linear calibration curve of rufinamide (Area vs Concentration / μg/ml^{-1})

2.3.4 Preparation of standard solution and test solution

Standard and test stock solutions (50 ppm) of rufinamide were prepared using 100 mg of standard and test sample of rufinamide. Drugs was dissolved in 25 mL sof methanol and sonicated for 10 min. Then the volume was made up to 100 mL with diluent. After that 5mL of above solution was diluted up to 100 mL with methanol.

2.3.5 Chromatographic Conditions

Optimisation of chromatographic conditions

The mobile phase for the proposed method (Phosphate buffer pH 4 : Acetonitrile 60 : 40) was filtered through 0.45 µm membrane filter. It was degassed with a sonicator for 15 min and pumped from the reservoir to the column (Phenomenex C-18, 250 mm × 4.6 mm, 5µm) at aflow rate of 1mL

Standard

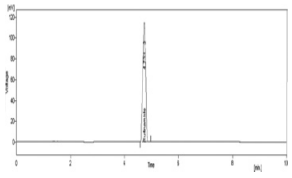


Figure 2

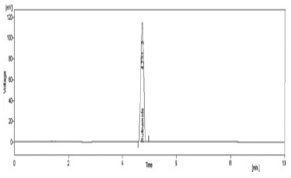


Figure 3

Sample Chromatogram

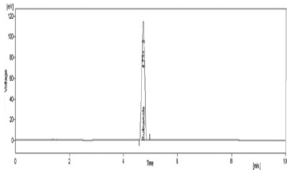


Figure 4

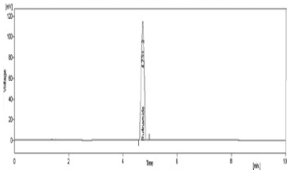


Figure 5

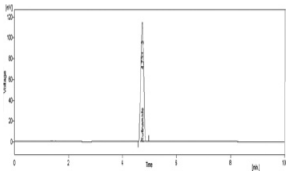


Figure 6

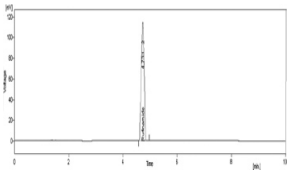


Figure 7

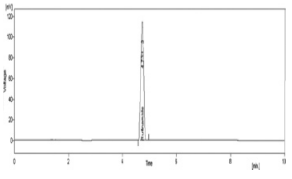


Figure 8

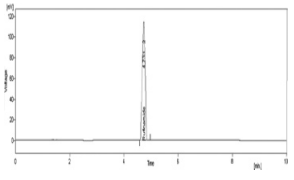


Figure 9

min^{-1}. The run time was set at 10 min. Prior to injection of the drug solutions the column was equilibrated for at least 1 h with mobile phase flowing through the system.The analyte was monitored at 225 nm and data acquired was stored and analyzed with spinchrom CFR chromatography software.

2.4 Method Validation Studies

Selectivity of the method was assessed on the basis of elution of Rufinamide using the above mentioned chromatographic conditions. The linearity, precision, accuracy, limit of detection, limit of quantitation and robustness has been validated for the determination of Rufinamide**.**

2.4.1 System precision

Six replicate injections of standard solution were given and mean of all of these values gives rise to the RSD value obtained. According to USP %RSD (Relative Standard Deviation) should not be more than 2% (Riley, M., Rosanke, T.W., 1996).

2.4.2 Method Precision

Method precision or Intra-assay precision data were obtained by repeatedly analyzing, in onelaboratory on one day, aliquots of homogeneous sample, each of which were independently prepared according to method procedure (Sethi, P.D., 2001 and Kar, A., 2005).

2.4.3 Linearity

Linearity of method is determined in the range 50-150 µg mL^{-1} (50%-150%). According to International Conference on Harmonisation (I.C.H) guidelines correlation coefficient should be less than 0.999 (ICH, 1996).

2.4.4 Ruggedness

This analysis was repeated with different column on different day with different analyst and different system and %RSD value was determined.

2.5 Stability in Analytical Solution

A drug solution of 50 ppm was prepared and kept at room temperature i.e. 25°C for 24 hrs. After that drug solution was analyzed and it was found to be stable at room temperature (Chafez, L., 1971).

2.5.1 Degradation studies of Rufinamide

The drug was allowed to degrade in acidic, basic, oxidative and thermal conditions (Chafez, L., 1971).

3. RESULTS AND DISCUSSION

3.1 System precision

The system precision was analyzed by six replicate injections each of standard solutions of rufinamide (50 ppm) into the HPLC system and the results are presented in Table 2. Percentage RSD for system precision was found to be 0.75%.

Table 2: Different trials carried out for developing the current HPLC

S. No	Column Used	Mobile Phase	Mode	Injection Vol.	Observation	Result
6.1	Phenomenex luna^{R} C18 (4.6 × 250) mm, 5µm	Ammonium acetate (pH-6.7) : Can (60 : 40)	Isocratic	20µl	Peak shape was not good	Method rejected
6.2	Phenomenex luna^{R} C18 (4.6 × 250) mm, 5µm	Ammonium acetate (pH-6.7) : Can (55 : 45)	Isocratic	20µl	Peak shape was not ok (Fronting)	Method rejected
6.3	Phenomenex luna^{R} C18 (4.6 × 250) mm, 5µm	Ammonium acetate (pH-2.5) : ACN : Methanol (60 : 30 : 10)	Isocratic	20µl	Peak width was more (peak shape was not good)	Method rejected
6.4	Phenomenex luna^{R} C18 (4.6 × 250) mm, 5µm	Triethylamine (pH-3) : ACN (70 : 30)	Isocratic	20µl	Extra peak as interferences were present	Method rejected
6.5	Phenomenex luna^{R} C18 (4.6 × 250) mm, 5µm	Triethylamine (pH-3) : ACN (50 : 50)	Isocratic	20µl	Tailing was more and absorbance was less	Method rejected
6.6	Phenomenex luna^{R} C18 (4.6 × 250) mm, 5µm	Triethylamine (pH-3) : Methanol (80 : 20)	Isocratic	20µl	More run time, peak shape was not good	Method rejected
6.7	Phenomenex luna^{R} C18 (4.6 × 250) mm, 5µm	Triethylamine (pH-4) : Methanol (75 : 25)	Isocratic	20µl	peak shape was not good	Method rejected
6.8	Phenomenex luna^{R} C18 (4.6 × 250) mm, 5µm	Buffer (Na_2HPO_4+ KH_2PO_4) (pH-4) : ACN (50 : 50)	Isocratic	20µl	Peak shape was not good	Method rejected
6.9	Phenomenex luna^{R} C18 (4.6 × 250) mm, 5µm	Buffer (Na_2HPO_4+ KH_2PO_4) (pH-4) : ACN (60 : 40)	Isocratic	20µl	Extra peaks as interference were present	Method rejected
6.10	Phenomenex luna^{R} C18 (4.6 × 250) mm, 5µm	Buffer (Na_2HPO_4 + KH_2PO_4) (pH-4) : ACN (60 : 40)	Isocratic	20µl	Both peak shape and absorbance were good	Method accepted

3.2 Method Precision

Two injections of standard solutions of rufinamide (50 ppm) were injected to check the system suitability. Then six sample rufinamide each batch were prepared separately andinjected in duplicate. Results are presented in Table 3, then %RSD for method precision was found to be 0.77%.

Table 3: Six replicate injection of the stock solution (50ppm)

Injection	Area counts/mV*sec
1	1324
2	1342
3	1350
4	1348
5	1332
6	1333
Mean	1338
SD	10
%RSD	0.75

3.3 Linearity

Linearity was determined by injecting six replicate injections of standard solutions of rufinamide (50 ppm) to check the system suitability. Then, the different concentration of sample solution was injected in duplicate in the concentration range of 50%–150% of drug substance, and the results are presented in Table 3. The correlation coefficient was found to be 0.999 from six replicate injections.

3.4 Ruggedness

Analysis was carried out with different analyst, using different column and different day and the results are presented in Table 4. The %RSD for ruggedness was found to be 0.44%.

3.5 Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. Such as change in flow rate (± 10%), pH (± 0.2 units) and organic content (± 2%). The results are presented in Table 6.9- Table 6.17. along with system suitability parameters of normal methodology.

Table 4. Method precision for calibration data Rufinamide

Type of Sample	Weight/mg	Inj-1 (Area)	Inj-2 (Area)	Mean area counts/mV*sec	Assay/% w/w^{-1}
Standard	50.16	1324	1342	1333	
Sample 1	50.16	1338	1348	1343	99.82
Sample 2	50.02	1340	1336	1338	99.72
Sample 3	50.85	1346	1339	1343	98.46
Sample 4	50.16	1340	1364	1352	100.49
Sample 5	50.12	1350	1346	1348	100.27
Sample 6	49.98	1348	1346	1347	100.48
				Mean Assay/ %w/w^{-1}	99.87
				SD	0.765
				% RSD	0.77

Standard

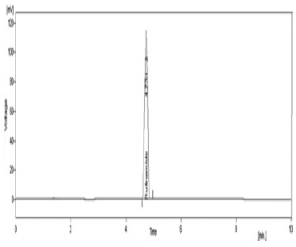


Figure 10

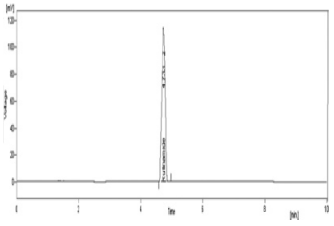


Figure 11

Sample Chromatogram

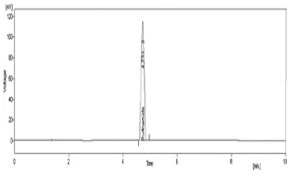


Figure 12

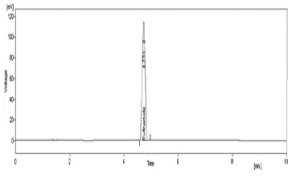
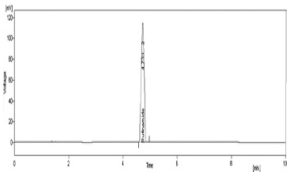


Figure 13



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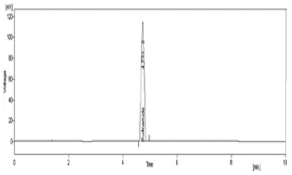

Figure 15

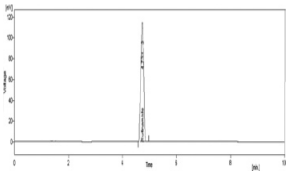

Figure 16

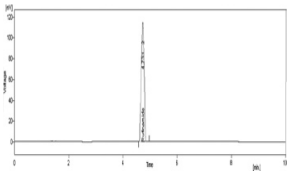

Figure 17

Table 5. Rufinamide Ruggedness Assay

Type of Sample	Weight/mg	Inj-1 (Area)	Inj-2 (Area)	Mean area counts/V*sec	Assay/% ww^{-1}
Standard	50.26	1358	1372	1365	99.87
Sample 1	50.06	1359	1368	1368	99.98
Sample 2	50.02	1358	1362	1362	99.77
Sample 3	50.04	1363	1367	1367	99.14
Sample 4	50.03	1348	1359	1359	99.31
Sample 5	50.06	1352	1366	1366	99.61
Sample 6	50.05	1364	1353	1353	99.63
				Mean Assay/ $\%ww^{-1}$	99.63
				SD	0.473
				% RSD	0.44

3.6 Stability in Analytical Solution

The sample was found to be stable at 25ºC for 24 hrs and the overall %RSD was found to be 0.45 and the result are presented in Table 7.

Table 6: Robustness parameter

Parameter	% RSD	%Assay
Increased Wavelength (+5)	0.74	98.88
Decreased Wavelength (-5)	0.64	98.86
Increased pH (- 0.2)	0.69	99.38
Decreased pH (+ 0.2)	0..69	99.65
Increased Flow rate (+0.1ml)	0.84	98.79
Decreased Flow rate (-0.1ml)	0.77	99.59
Organic content increased	0.66	99.58
Organic content decreased	0.71	99.19

Table 7: Stability data for Rufinamide (at 25°C):

S.NO.	Time	Interval/ hrs	Interval / min	Area counts/ µv*sec	Cumulative RSD(%)	Assay
1	6/8/2010 14:00			1338	0.11	99.55
2	6/8/2010 14:30	0 : 30 : 00	30	1340	0.15	99.70
3	6/8/20103:00:00 PM	1 : 00 : 00	60	1336	0.46	99.41
4	6/8/2010 17:00	3 : 00 : 00	180	1350	0.61	100.45
5	6/8/2010 20:00	6 : 00 : 00	360	1355	0.69	100.82
6	6/9/2010 8:00	18 : 00 : 00	1080	1330	0.69	98.96
7	6/9/2010 14:00	24 : 00 : 00	1440	1332	0.45	99.11
				Mean% RSD	0.45	99.71

3.7 Degradation studies of Rufinamide

The drug was allowed to degrade in acidic, basic, oxidative and thermal conditions and results are presented in Table 8. There was no co eluting peaks (Figure 18).

4. RESULTS

Figure 2 shows typical chromatogram of Rufinamide. System stability tests were carried out on freshly prepared standard stock solutions of Rufinamide at 25°C (Table 5). The calibration curve was linear in the range of 50-150 µg/ml for rufinamide. The degradation studies of Rufinamide are shown in Table 6.

Table 8: Degradation studies of Rufinamide:

Degradation studies	% degradation
Alkaline degradation (0.05 N NaOH, 1 h)	3
Alkaline degradation (0.01 N NaOH, 1 h)	7
Alkaline degradation (1 N NaOH, 1 h)	13
Alkaline degradation (2 N NaOH, 1 h)	15
Alkaline degradation (5 N NaOH, 1 h)	25
Acidic degradation (0.1 N HCl)	16
Oxidative degradation (1% H_20_2)	11
Oxidative degradation (3% H_20_2)	20
Thermal degradation (solid sample, 100 °C, 24 h)	0
Thermal degradation (solid sample, 100 °C, 24 h)	0

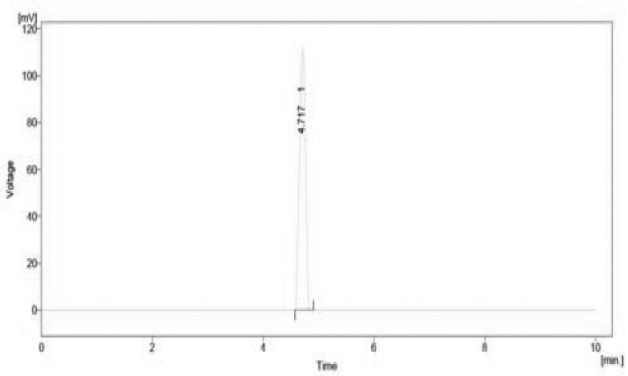

Figure 18. Chromatogram of Rufinamide. (The peak retention time of Rufinamide was 4.717 min)

5. CONCLUSION

HPLC method was successfully developed and validated for determination of rufinamide. The total run time was 10 min. Method validation results have proved the method to be selective, precise, accurate, robust and stability indicating. Thus, the developed stability indicating assay method can be successfully applied for routine analysis of rufinamide.

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