

## A Stability Indicating Liquid Chromatography Method for the Assay of Rufinamide Bulk Material and Tablets

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**A simple, rapid, isocratic stability indicating reverse phase liquid chromatography method was developed for the assay of rufinamide bulk drug and tablets. The method achieved adequate resolution of rufinamide, related substances A and B as well as laboratory generated degradation products. The method uses a Phenomenex® Hyperclone BDS C-18 column (250 × 4.6 mm, 5 μ) maintained at 35 °C and a mobile phase composed of methanol-0.1 M octane sulfonic acid-0.1 M KH<sub>2</sub>PO<sub>4</sub>, pH 6.5-water (30:10:5:55, % v/v/v/v) delivered at a flow rate of 1.0 ml/min. The eluents were monitored by means of ultraviolet detection at 210 nm. During validation, the method satisfied the International Conference on Harmonization acceptance criteria for linearity sensitivity, precision, accuracy, and robustness. The developed method may be applied in the routine analysis of rufinamide bulk material and tablets as well as stability studies.**

**Key words:** Rufinamide, stability indicating method, related compound

### INTRODUCTION

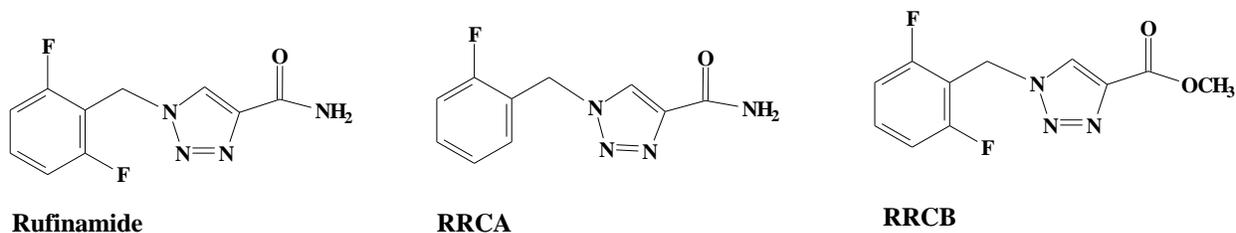
Rufinamide (Figure 1) is a triazole anticonvulsant used in conjunction with other therapies to manage seizures associated with Lennox-Gastaut Syndrome (LGS) and various other seizure disorders in adults, as well as in children aged >4 years [1, 2]. Lennox Gastaut Syndrome is a rare severe type of epilepsy that is refractory to traditional antiepileptic drugs such as hydantoins, barbiturates, phenyltriazines, benzodiazepines, gamma-aminobutyric acid (GABA) analogs, dibenzazepines and carbamates [3-8]. Rufinamide is a new anticonvulsant drug which has revolutionized the management of LGS [9-10]. The compound is associated with related substances arising from chemical synthesis [11].

Although rufinamide is chemically stable, it generates minor degradation products under stress conditions such as acidic or alkaline solutions, oxidative, photolytic and thermal degradation [2]. Therefore, there is need to develop suitable high performance liquid chromatography (HPLC) methods for the

determination of rufinamide in the presence of its related substances and degradates. The United States Pharmacopeia (USP) defines two related compounds of rufinamide designated A and B (Figure 1) [12].

The USP specifies a HPLC method using a C-18 column and a mobile phase composed of methanol - THF - KH<sub>2</sub>PO<sub>4</sub> buffer mixture for the assay of rufinamide [12]. This method suffers the drawback of utilizing tetrahydrofuran (THF) in a binary organic phase and high buffer content in the mobile phase which undermines the longevity of the column and chromatography equipment. In addition, several reverse phase HPLC (RP-HPLC) methods for the assay of rufinamide formulations have been reported though not stability-indicating [1, 13-14]. Annapurna *et al.* (2012) and Patel *et al.* (2014) published stability indicating RP-HPLC methods for rufinamide but did not demonstrate synchronized separation of synthetic route related substances [2, 15]. This paper reports on a simple, rapid and isocratic stability RP-HPLC method for the separation of rufinamide, degradation products and related substances.

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**Figure 1: Chemical structures of rufinamide and rufinamide related compounds RRCA and RRCB**

## EXPERIMENTAL

### Chemicals

Analytical grade  $\text{KH}_2\text{PO}_4$  (Loba Chemie Pvt Ltd, Mumbai, India),  $\text{K}_2\text{HPO}_4$  (RFCL Ltd, New Delhi, India) and sodium octane sulfonate (Oxford Lab Chem, Maharashtra, India) were used in the preparation of buffer solutions. Analytical grade NaOH pellets, concentrated HCl (Loba Chemie Pvt Ltd, Mumbai, India) and 6% v/v hydrogen peroxide solution (Oxford Lab Chem, Maharashtra, India) were employed in the forced degradation experiments. Methanol (Scharlau, Barcelona, Spain) was of HPLC grade. Purified water was prepared in the laboratory by means of an Aquatron Automatic Water Stills A4000 (Bibby Scientific Ltd, Staffordshire, UK).

### Instrumentation

A Shimadzu Prominence manual HPLC system (Shimadzu Corporation, Kyoto, Japan) supported by a CBM 20A (S/N: L20234505098) Prominence communication bus module system controller and a LC Solution software Ver. 1.22, SP1 equipped with an SPD-20A (S/N: L20134506368) Prominence UV/Visible detector using a deuterium lamp for ultraviolet and a tungsten lamp for visible detection was used. A LC-20AT (S/N: L2011450625) Prominence solvent delivery system with a dual-plunger tandem-flow solvent delivery module and a DGU-20A<sub>3</sub> (S/N: 20254405376) Prominence degasser were part of the HPLC system. The temperature was controlled using a CTO-10AS VP (S/N: 21044505694) column oven with a block heating thermostatic chamber and a preheater system.

### Mobile phases

Buffer solutions (0.1 M) were prepared by mixing equimolar (0.1 M) solutions of potassium phosphate and ion pairing agent (0.1M) before adjusting the pH. Mobile phases were made by diluting the buffer solutions with water before topping up with methanol. The mobile phases were degassed using a power sonic 410 bench top ultrasonic bath (Daihan Labtech Ltd, Kyonggi-Do, Korea). Standard and sample solutions were dissolved in a diluent consisting of acetonitrile-water (60:40).

### Reference standards and samples

Rufinamide reference standard (99.7% w/w), rufinamide related compound A (RRCA) and rufinamide related compound B (RRCB) working reference standards were purchased from United States Pharmacopeial Convention (Rockville, MD, USA). Inovelon<sup>®</sup> 200 mg tablets were purchased from United Kingdom while the other samples rufinamide 200 mg tablets were prepared in the laboratory.

### Working reference solution

The working reference solution (WRS) was used for optimization of chromatographic conditions. For this purpose, rufinamide standard (25 mg) and 62.5 mg each of RRCA and RRCB were weighed into a 50 ml VF. Additionally, 100 mg of rufinamide was separately subjected to degradative stress conditions in 50 ml of the following solutions; 1.0 M NaOH and 3.0% v/v  $\text{H}_2\text{O}_2$ . Acidic conditions did not yield in detectable degradation. Aliquots of each of the degradation reaction mixtures (0.1 ml) were individually added to the 50 ml VF and the

solution was made to volume with the diluent. A 4 ml aliquot of the resulting solution was diluted to 25 ml VF to obtain concentrations of rufinamide 0.08 mg, RRCA 0.2 mg and RRCB 0.2 mg per ml.

### Sample solutions

Twenty tablets of each product were pulverized into a fine powder whereof weight of powder equivalent to 25 mg of rufinamide was transferred into a 50 ml volumetric flask. Acetonitrile (20 ml) was added and the mixture sonicated for 15 min before making to volume with acetonitrile. An aliquot (4 ml) of the resulting solution were transferred into a 25 ml volumetric flask and diluted to volume using the diluent to yield a concentration of 0.08 mg/ml, sonicated for 15 min, filtered through a 0.45  $\mu$  membrane filter and 20  $\mu$ l injected into the HPLC system.

### Method validation

**Rufinamide standard solution:** Rufinamide standard solution (RSS) was prepared to a concentration of 1 mg ml<sup>-1</sup> solution in diluent. This solution (corresponding to 100%) was used for linearity, range, precision and sensitivity tests.

**Linearity and range:** Rufinamide solutions were prepared at concentrations equivalent 0.4, 0.6, 0.8, 1.0 and 1.2 mg ml<sup>-1</sup> in diluent. The resulting solutions were analyzed in triplicate, the peak areas normalized and treated to a 6-point linearity regression curve. The slope, y-intercept and coefficient of determination ( $r^2$ ) were used as measure of linearity.

**Precision:** Repeatability was determined by making 6 injections of the RSS on the same day. The coefficient of variation (CV) of the peak areas of rufinamide was computed. For intermediate precision, 6 replicate injections of a freshly prepared RSS were run on 3 consecutive days using freshly prepared mobile phases. The peak areas obtained were normalized and the CV thereof used to evaluate the inter-day precision of the method.

**Sensitivity:** The limit of detection (LOD) and limit of quantitation (LOQ) were determined by preparing serial dilutions of the RSS. The signal to noise ratio (S/N) values of the rufinamide peak were determined with reference to the diluent as blank. The LOD was derived from the lowest concentration of the analytes that yielded S/N of 3:1 while S/N 10:1 coupled with a peak area CV of 10-20% were used to establish LOQ [16–18].

**Robustness:** The influence of buffer pH, temperature and methanol on separation was tested at 3 levels, low (-1), central (0) and high (1) as shown in Table 1. The WRS was run under the different factor levels and the capacity factors ( $k'$ ) of component peaks determined. The  $k'$  was plotted against each factor series for assessment of selectivity.

**Accuracy:** The accuracy for the developed method was determined by spiking a placebo mixture with rufinamide working standard and determining the recovery upon analysis. The recovery experiments were carried out at the 80, 100 and 120 % levels. The percentage recovery was used as a measure of accuracy according to ICH guidelines [16–18].

**Table 1: Robustness testing levels for the chromatographic factors**

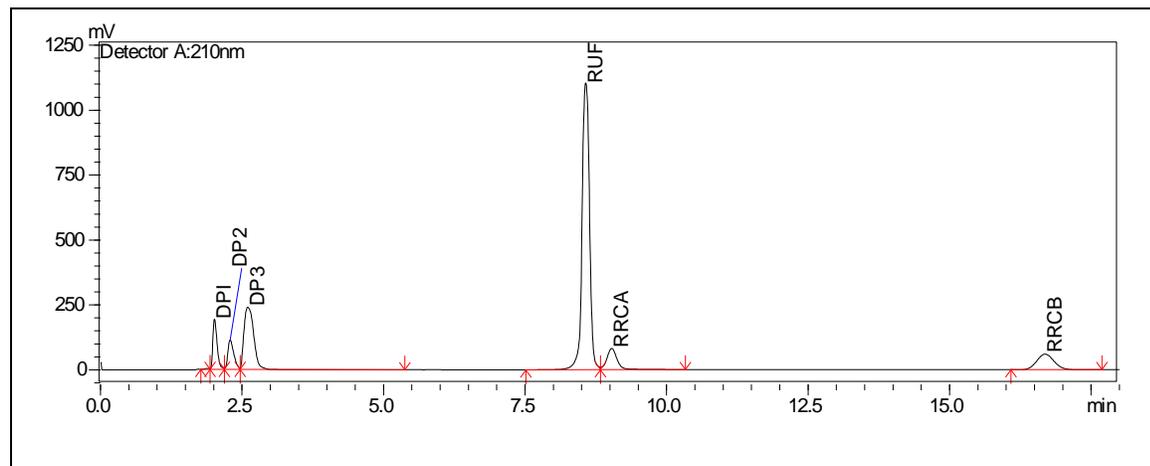
Factor level	pH	Temperature (°C)	Acetonitrile concentration (%v/v)
1	7.0	35	45
0	6.5	30	40
-1	6.0	25	35

## RESULTS AND DISCUSSION

### Optimization of chromatographic conditions

Preliminary experiments aided the choice of the column, flow rate, detection wavelength and organic modifier. Potassium phosphate buffer system was selected on account of suitable peak parameters and the low wavelength of detection selected. The peaks corresponding to rufinamide (RUF) and rufinamide related compound A (RRCA) were designated the critical peak pair (CPP) for optimization of the factors owing to their poor separation character. When the influence of pH (5-7) on the separation of the component peaks was examined, resolution of the CPP occurred at pH 6.5 which was

consequently taken as optimum. Methanol concentration was investigated within the range 25-40% v/v whereof separation of the CPP was attained at 30%. Incorporation of sodium octane sulfonate as ion pairing agent reduced retention times of the component peaks and improved selectivity at a concentration of 10 mM. Systematic evaluation of the temperature and buffer concentration yielded the optimum chromatographic conditions as: a mobile phase consisting of methanol-0.1M octane sulphonic acid-0.1M  $\text{KH}_2\text{PO}_4$ , pH 6.5-water (30:10:5:55, % v/v/v/v) delivered at  $1.0 \text{ ml min}^{-1}$ . The column temperature was set at  $35 \text{ }^\circ\text{C}$  while the eluents were monitored at a wavelength of 210 nm. Figure 2 is a typical chromatogram obtained under optimum conditions.



**Figure 2. Typical chromatogram at optimized conditions.**

Column: Hyperclone BDS C18,  $5\mu$ ,  $250 \times 4.6 \text{ mm ID}$ ; Temperature,  $40 \text{ }^\circ\text{C}$ . Mobile phase: methanol-0.1M octane sulfonic acid-0.1M  $\text{KH}_2\text{PO}_4$ , pH 6.5-water (30:10:5:55, % v/v/v/v). DP1-4: degradation products 1-4. RUF: rufinamide. RRCA and RRCA, rufinamide related compounds A and B respectively.

### Method validation

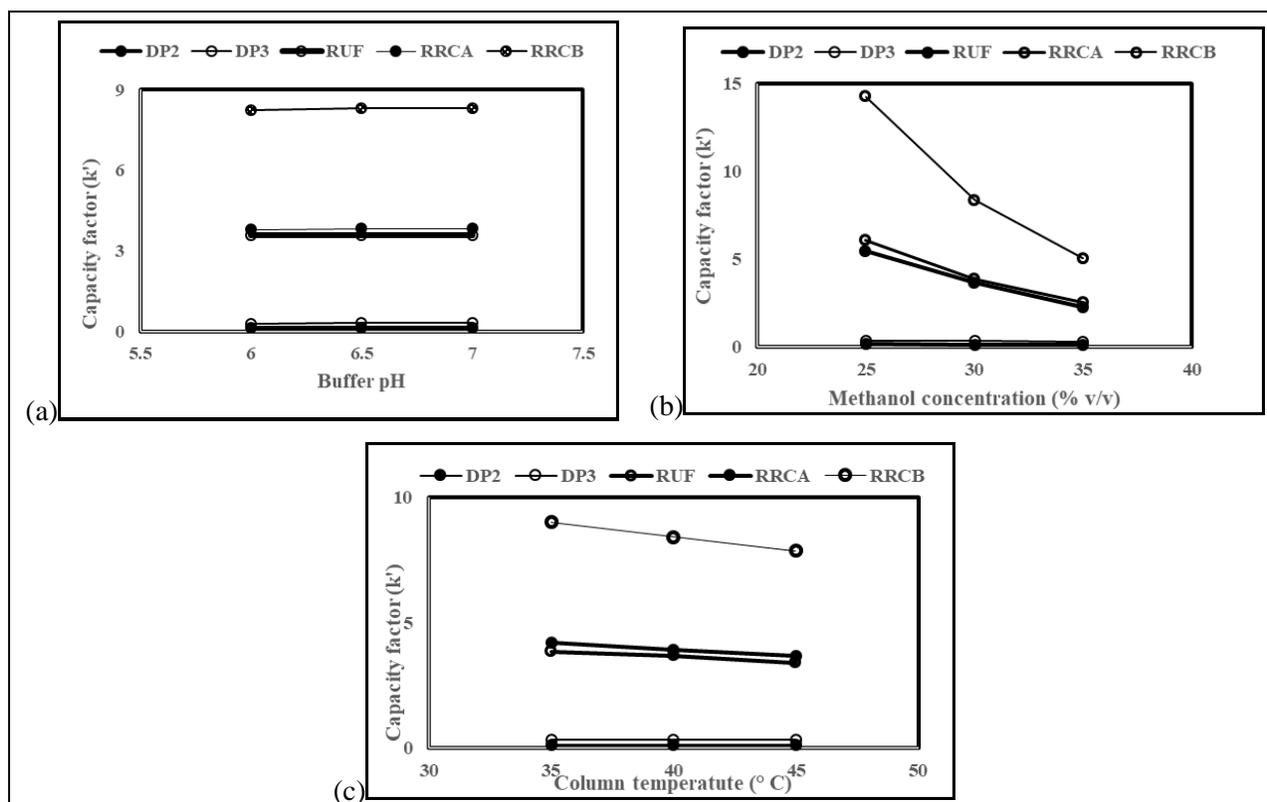
The validation results for rufinamide are shown in Table 2. There was a linear relationship between concentration and peak area within the 25-150% range with  $r^2 < 0.999$ . The repeatability and inter-day variation of the peak areas satisfied the ICH acceptance criteria. The average percentage recovery of rufinamide was 99.5 % thus indicating acceptable accuracy of

the method. Figure 3 shows the effect of pH, temperature and methanol concentration on the  $k'$  of the peaks as measure of selectivity during the robustness experiments. Methanol concentration exhibited the greatest impact on capacity factors especially the DP3 peak. Temperature and pH changes did not considerably alter the retention of the analytes. In all cases the resolution of the CPP was maintained above 2.0 while the CV of the peak areas was 0.11-0.86.

**Table 2 Method validation results**

Parameter	Linearity ( $y = ax + b$ )*	LOD (ng)	LOQ (ng)	Repeatability (CV)	Inter-day precision (CV)	Average recovery
Value	$a = 23977897$ $b = 23171717$ $r^2: 0.9997$	156	311	0.9	0.9	99.5 CV = 0.6
Acceptance criteria [16-18]	$r^2 > 0.9990$	-	CV < 2 0	CV < 1	CV < 3	98-102% CV < 5

\*Key: a = slope, b = intercept.

**Figure 3: Effect of variation of the buffer pH (a), methanol concentration (b) and column temperature (c) on capacity factors of the analytes.****Analysis of samples**

The developed method was applied in the analysis of rufinamide tablets. Inovelon<sup>®</sup> and three batches of the laboratory generated tablets with a label claim of 200 mg of rufinamide were

subjected to analysis. The results obtained are summarized in Table 3. The results of the assay indicate the method is selective for the assay of rufinamide without interference from the excipients. None of the related substances was detected in the samples.

**Table 3: Assay results for rufinamide tablets**

Product	Inovelon <sup>®</sup>	F1	F2	F3	F4
Rufinamide content as % label claim	99.7 (0.5)	97.1 (1.5)	104.2 (0.6)	98.1 (1.9)	98.2 (0.5)

F1-F4 represent four laboratory batches of rufinamide tablets. Figures in parentheses represent the CV, n = 3

## CONCLUSION

A stability indicating RP-HPLC method was developed for the analysis of rufinamide bulk material and dosage formulations. The method can be applied in the routine analysis of rufinamide samples as well as in stability studies.

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