STM JOURNALS

Emerging Trends in
Personalized Medicines

Volume 1, Issue 1, 2024
January-June
(Journal):10.37591/ETPM
DOI: 10.37591/ETPM

https://journals.stmjournals.com/etpm

Research ETPM

Development and Validation of a Robust RP-HPLC Method for Rufinamide Quantification in Pharmaceutical Dosage Forms

Thonta Harini Kumari[1,*], Shaik Ejas[2]

Abstract

The creation and validation of a simple, precise, and targeted reverse phase high-performance liquid chromatographic (RP-HPLC) approach allowed for the quantification of rufinamide in pharmaceutical dosage forms. With a mobile phase of methanol and water (50:50, v/v), the technique used a gradient mode Symmetry Qualisil gold C18 column (4.6 × 150mm, 5 µm). For the detection method, a flow rate of 1 mL/min was employed at 220 nm. During a 5.203-minute retention period, the primary peak for rufinamide was detected. Linearity, accuracy, precision, robustness, ruggedness, limit of quantitation (LOQ), robustness, and precision were among the criteria that were evaluated during the method's validation. With a correlation value (r) that was almost equal to unity, the method demonstrated good linearity in the concentration range of 10 to 60 mcg/ml. The sensitivity of the method was demonstrated by the determination of the LOD and LOQ for rufinamide, which were found to be 1.51 mcg/mL and 4.60 mcg/mL, respectively. The method's repeatability and reproducibility were demonstrated by precision studies, which showed low relative standard deviation (RSD) values for both intra-day and inter-day analysis. Recovery experiments at three distinct concentration levels, which produced recovery values ranging from 99.08% to 100.93%, validated the accuracy of the procedure. By purposefully altering the experimental settings and chromatographic conditions, the robustness and roughness of the method were assessed, proving its dependability and appropriateness for regular analysis. In summary, the new RP-HPLC method provides a precise, accurate, and dependable way to measure rufinamide in pharmaceutical formulations. Due to its simplicity and specificity, it can be used in pharmaceutical enterprises for routine quality control analyses that guarantee the potency and purity of dosage forms containing rufinamide.

Keywords: Rufinamide, RP-HPLC, pharmaceutical dosage forms, method validation, sensitivity

INTRODUCTION

Triazole derivative rufinamide is a key pharmacological intervention for the treatment of seizure disorders, including partial seizures and Lennox-Gastaut syndrome. It is a crucial weapon in the fight against epilepsy due to its therapeutic effectiveness and mode of action. This paper explores the molecular basis, therapeutic uses, and pharmacological aspects of rufinamide.

*Author for Correspondence
Thonta Harini Kumari
E-mail: thontaharinikumari@gmail.com

[1]Student, Department of Pharmaceutical Analysis, Mak college of Pharmacy, Moinabad, Hyderabad, Telangana, India.
[2]Associate Professor, Department of Pharmaceutical Analysis, Mak college of Pharmacy, Moinabad, Hyderabad, Telangana, India.

Received Date: March 02, 2024
Accepted Date: March 12, 2024
Published Date: March 20, 2024

Citation: Thonta Harini Kumari, Shaik Ejas. Development and Validation of a Robust RP-HPLC Method for Rufinamide Quantification in Pharmaceutical Dosage Forms. Emerging Trends in Personalized Medicines. 2024; 1(1): 25–32p

The path taken by rufinamide from its discovery to its use in clinical settings highlights a collaborative effort combining pharmacology, chemistry, and clinical medicine. Rufinamide is structurally classified as a derivative of triazoles, and its IUPAC designation provides information about its chemical makeup: The IUPAC

designation is 1-[methyl (2,6-difluorophenyl)].1-H-1,2,3-triazole-4-carboxamide. $C_{10}H_8F_2N_4O$ is the molecular formula. There is 238.1 molecular weight [1, 2]. The basis for its pharmacological activities is embodied in this molecular structure shown in Figure.1, which is made up of atoms of carbon, hydrogen, fluorine, nitrogen, and oxygen [3, 4].

The voltage-gated sodium channel (VGSC) interaction is the main focus of rufinamide pharmacokinetics.These channels control the influx of sodium ions during action potentials, which is a critical aspect of neuronal excitability. To stabilize neuronal membranes and prevent seizure activity from spreading, rufinamide works by keeping VGSCs inactive for longer periods of time. Rufinamide lowers the frequency and intensity of epileptic seizures by modifying sodium channel kinetics, which is how it produces its anticonvulsant effects.

The effectiveness of rufinamide in the treatment of a variety of seizure disorders has been confirmed by clinical trials and empirical data. The treatment effectiveness of this medication is demonstrated by its ability to treat Lennox-Gastaut syndrome, a severe type of childhood epilepsy marked by cognitive impairment and numerous seizure types. Further increasing rufinamide's clinical value across a range of epileptic diseases, it also shows promise in treating partial seizures.

Apart from its medicinal effectiveness, rufinamide's pharmacokinetic characteristics are worth considering. Rufinamide is rapidly absorbed when taken orally, peaking in plasma concentrations in a few hours. Its broad metabolism produces active metabolites that contribute to its anticonvulsant actions, which are mostly mediated by hepatic enzymes. By maximizing therapeutic effectiveness and minimizing side effects, dose optimization and treatment monitoring are made easier with an understanding of rufinamide's pharmacokinetic features.

Priority one while using rufinamide in clinical settings should be given to both safety and tolerability. Common side effects, albeit usually easily managed, include lightheadedness, sleeplessness, and gastrointestinal issues. Adverse events are rare but dangerous; during medication, close observation is required for reactions such hypersensitivity reactions and haematological abnormalities. While customising treatment plans for each patient, clinicians must weigh the therapeutic advantages of rufinamide against any possible hazards.

Figure 1. Structure of Rufinamide.

A review of the literature found that there aren't many techniques available for analysing rufinamide, either by itself or in combination with other medications, in both pure form and pharmaceutical formulations [5–11]. Because routine analysis of rufinamide estimate in pharmaceutical dose form requires an appropriate, affordable RP-HPLC technology. An analytical approach for estimating rufinamide that is straightforward, accurate, precise, and economical has been attempted. According to ICH guidelines, we will validate the proposed method. The goal of the proposed work is to use RP-HPLC to establish a new, straightforward, sensitive, accurate, and cost-effective analytical method and validation for the determination of rufinamide in pharmaceutical dosage form.

MATERIALS AND METHODS

During this study, we have created a straightforward and accurate RP-HPLC method for quantifying rufinamide in pharmaceutical and bulk drug formulations.

Experimental

Instrumentation

EZ Chrome Elite software was utilised in conjunction with an Auto Sampler, DAD or PDA detector, and isocratic high performance liquid chromatography system. A 4.6 x 150 mm, 5 µm Qualisil gold C18 Column was utilized.

Reagents

Ranbaxy Laboratories graciously donated the reference standard rufinamide (Ahmadabad, India). No additional purification was required before using the regular medications. Throughout the trial, Qualigens in Mumbai provided methanol and additional HPLC-grade reagents. The mobile phase consists of a 50:50 (v/v) mixture of water and methanol.

Preparation of Working Stock Solution of Rufinamide

Weigh and transfer 10 mg of rufinamide precisely. Fill a 10 mL volumetric flask with the working standard, and then add around 5 mL of diluent. Sonicate to fully dissolve the diluent, then add more solvent (stock solution) to bring the volume up to the mark. A 10 mL volumetric flask should be filled with 0.4 mL of the aforementioned stock solution, and then diluted with the diluent to the required volume. After thoroughly mixing, strain through a 0.45µm filter.

RESULTS AND DISCUSSION

Chromatographic Conditions

Equipment: Agilent High performance liquid chromatography equipped with Auto Sampler and DAD or PDA detector.

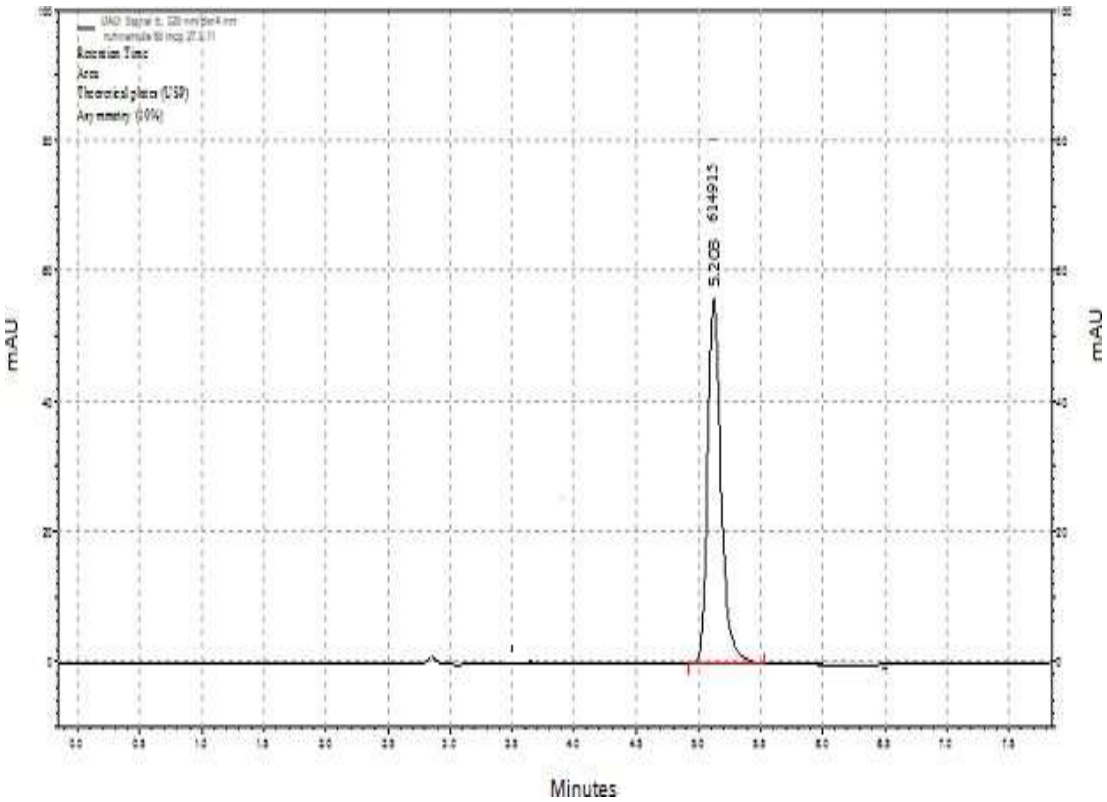

Figure 2. Standard chromatogram.

Column: Qualisil gold C18 (4.6 × 150 mm, 5 µm)
Flow rate : 1 ml/ min.
Wavelength : 220 nm Injection volume : 20 µl Column oven : Ambient
Run time : 8.0 min.

Assay procedure: By appropriately diluting the stock solution with the mobile phase, working standard solutions containing 10 to 60 mcg / ml of rufinamide were created. A 20 µl aliquot of every solution was injected into the column five times, and the chromatograms are shown in Figures 2–5 after being recorded. A retention time of 5.20 minutes was discovered. The calibration graph was created by graphing the average peak area in relation to the concentration of rufinamide shown in Table 1.

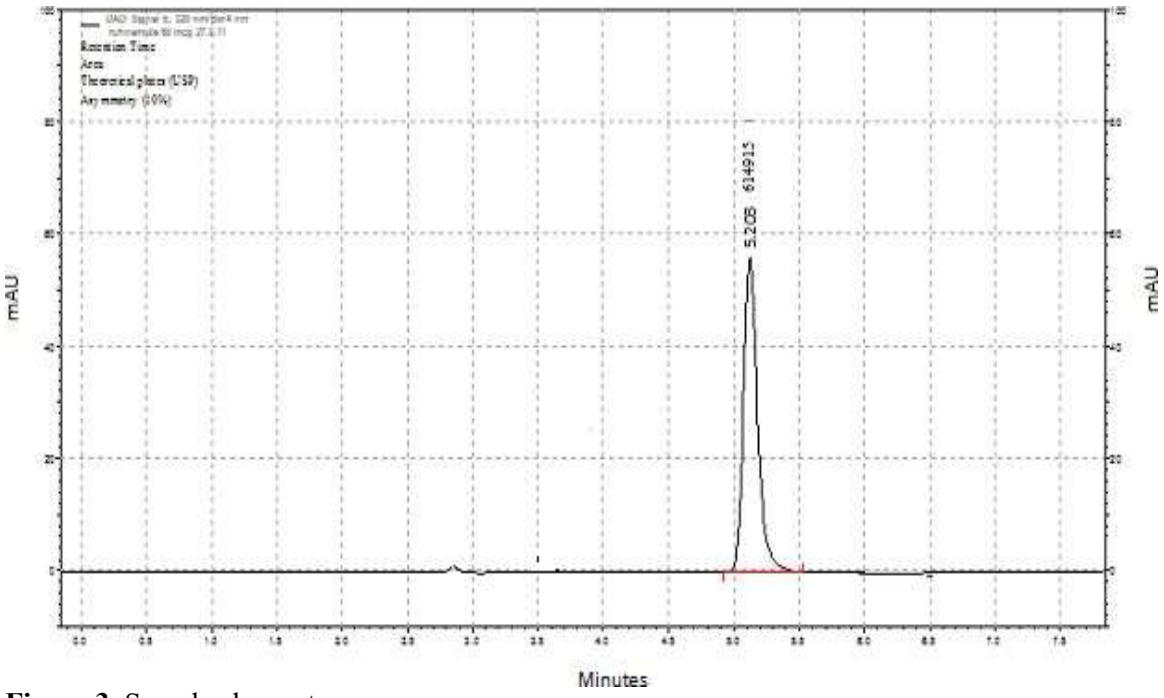

Figure 3. Sample chromatogram.

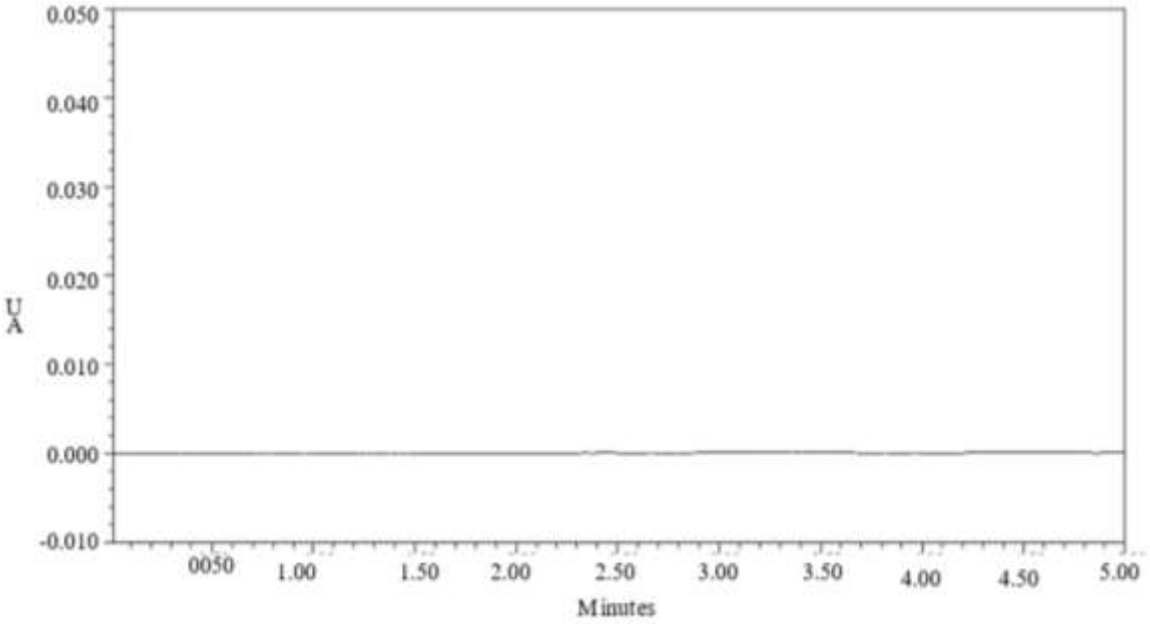

Figure 4. Blank chromatogram.

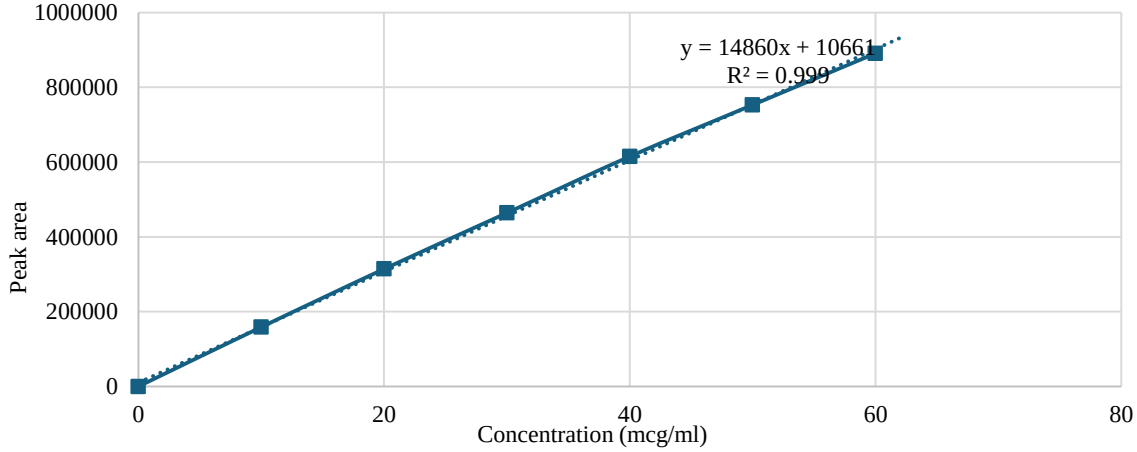

Figure 5. Linearity graph for Rufinamide.

Table 1. System suitability parameters.

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

Analysis of formulation: We carefully measured and finely powdered twenty tablets (Banzel). To achieve a concentration of 1000 mcg/ml, tablet powder equivalent to 0.01 mg of rufinamide was precisely weighed, dissolved in 50 ml of methanol in a 100 ml volumetric flask, and then diluted to the appropriate level with water. To obtain the required concentration of 40 mcg/ml by the RP-HPLC method, 0.4 ml of the aforementioned stock solution was added to a 10 ml volumetric flask and added to the mobile phase. The prepared combinations were filtered using Whatman filter paper. The chromatographic apparatus was injected with the final solution three times and shown in Table 2.

Table 2. Assay results for Rufinamide.

Tablet	Label claim (mg)	Analyst I		Analyst II	
		Amount found (mg)	*Recovery ± SD** (%)*	*Amount found (mg)*	*Recovery ± SD** (%)*
Banzel	400	399.87	99.87 ± 0.48	400.52	100.52 ± 0.204

Analytical Method Validation

Linearity: The linearity test was performed using concentrations ranging from 20 µg/mL to 100 µg/mL. A chromatographic system was injected with each level. The correlation coefficient was calculated using each level's area. Place each layer in a chromatographic instrument and measure the peak area. Generate a plot of concentration versus peak area, with peak area on the Y-axis and concentration on the X-axis, and calculate the correlation coefficient. Table 3 and Figure 5 shows results.

Table 3. Linearity results of Rufinamide.

S. N.	Conc. (mcg / ml)	Peak Area
1	10	158751
2	20	314117
3	30	464005
4	40	614915
5	50	752760
6	60	890650

Accuracy studies: The recovery study provided assistance in determining the accuracy. Three levels of recovery were used: 50%, 100%, and 150%. Put the standard solutions into the apparatus for chromatography. Determine the amount of rufinamide that was detected and added, as well as the values for the mean and individual recoveries. Table 4 shows the results.

Table 4. Showing accuracy results for Rufinamide.

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

Precision Studies: Six replicate injections of the standard were used to calculate precision using the coefficient of variation. The area was measured by HPLC after six dilutions of the standard solution. It was possible to find the %RSD for the six replicate injections. Table 5 shows results.

Table 5. Precision results for Rufinamide.

S.N.	Concentration (mcg / ml)	Intraday precision (Area)	Interday precision (Area)
1	40	610562	609231
2	40	614915	604938

3	40	609231	599231
4	40	599231	609231
5	40	604915	599231
6	40	599234	614915
Mean		606347.5	606129.5
Std.Dev		6368.985	6213.242
%RSD.		1.05	1.02

Ruggedness: Precision was carried out on a different day to assess the method's intermediate precision. The standard solution was diluted six times, and the area of each dilution was measured by HPLC. It was possible to find the %RSD for the six replicate injections. Table 6 shows the results.

Table 6. Ruggedness results of Rufinamide.

Tablet	**Label claim (mg)**	**Analyst I**		**Analyst II**	
		Amount found (mg)	***Recovery ± SD** (%)***	***Amount found (mg)***	***Recovery ± SD** (%)***
Sample	400	399.91	99.08 ± 0.48	400.21	100.28 ± 0.17

Robustness: Deliberately altering the Flow rate and Mobile Phase composition as part of the Robustness process allowed for the evaluation of the method's impact. Table 7 shows results.

Table 7. Robustness results for Rufinamide.

S.N.	**Change in flow rate**	**R.T.**
01	0.9 ml / min	5.8
02	1.1 ml / min	4.7

LOD and LOQ: LOD and LOQ were used to calculate the sensitivity of RP-HPLC. which, in accordance with ICH rules, were computed using the following equations from the calibration curve. Table 8 displays the results.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

Table 8. LOD, LOQ of Rufinamide.

Drug	**LOD**	**LOQ**
Rufinamide	2.82	10

CONCLUSION

After validation, the developed HPLC technique was determined to be straightforward, accurate, sensitive, and precise for estimating rufinamide in pharmaceutical dosage forms. Therefore, routine quality control analysis of rufinamide in pharmaceutical dosage forms can be carried out with ease and convenience using this technology.

REFERENCES

1. Arroyo S: Rufinamide. Neurotherapeutics. 2007 Jan;4(1):155–62.
2. Hakimian S, Cheng-Hakimian A, Anderson GD, Miller JW: Rufinamide: a new anti-epileptic medication. Expert Opin Pharmacother. 2007 Aug;8(12):1931–40.

3. Authors unspecified: Rufinamide: CGP 33101, E 2080, RUF 331, Xilep. Drugs R D. 2005;6(4):249–52.
4. Wier HA, Cerna A, So TY: Rufinamide for pediatric patients with Lennox-Gastaut syndrome: a comprehensive overview. Paediatr Drugs. 2011 Apr 1;13(2):97–106. doi: 10.2165/11586920–000000000–00000.
5. L.A. Brunner, M.L. Powell, an automated method for the determination of a new potential antiepileptic agent (CGP 33101) in human plasma using high performance liquid chromatography, Biomed. Chromatograph. 6 (6) (1992) 278–282.
6. M.C. Rouan, C. Souppart, L. Alif, et al., Automated analysis of a novel anti-epileptic compound, CGP 33,101, and its metabolite, CGP 47,292, in body fluids by high-performance liquid chromatography and liquid-–solid extraction, J. Chromatogr. B. Biomed. Appl. 667 (2) (1995) 307–313.
7. M.C. Rouan, C. Buffet, L Masson, et al., Practice of solid-phase extraction and protein precipitation in the 96-well format combined with high-performance liquid chromatography-ultraviolet detection for the analysis of drugs in plasma and brain, J. Chromatogr. B. Biomed. Sci. Appl. 754 (1) (2001) 45–55.
8. M. Contin, S. Mohamed, C. Candela, et al., Simultaneous HPLC-UV analysis of rufinamide, zonisamide, lamotrigine, oxcarbazepine monohydroxy derivative and felbamate in deproteinized plasma of patients with epilepsy, J. Chromatogr. B. Analyte. Technol. Biomed. Life Sci. 878 (3–4) (2010) 461–465.
9. M. Iolanda, R. Manuela, F. Cinzia, et al., Development and validation of an HPLC–UV detection assay for the determination of rufinamide in human plasma and saliva, Anal. Bioanal. Chem. 401 (3) (2011) 1013–1021.
10. M. Giancarlo la, M. Sabrina, F. Luca, et al., Rapid assay of rufinamide in dried blood spots by a new liquid chromatography– tandem mass spectrometric method, J. Pharm. Biomed. Anal. 54 (1) (2011) 192–197.
11. S. Muneer, C. Jose Gnana Babu, R. Hakeem, et al., Development and validation of RP-HPLC method for estimation of rufinamide in bulk and its pharmaceutical dosage form, Int. J. Pharm. Res. Anal. 2 (1) (2012) 9–13.

