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SHORT COMMUNICATION

Development and validation of a stability indicating RP-HPLC method for the determination of Rufinamide

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Abstract A stability-indicating RP-HPLC method was developed and validated for the determination of Rufinamide in tablet dosage forms using C 18 column (250 mm × 4.6 mm, 5 μm) with mobile phase consisting of water–acetonitrile (40:60, v/v) with a flow rate of 0.8 mL/min (UV detection 215 nm). Linearity was observed over the concentration range 1.0–200 μg/mL (R^2=0.9997) with regression equation y=113190 x+63053. Rufinamide was subjected to stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation. Rufinamide is more sensitive towards acidic degradation. The method was validated as per ICH guidelines.

1. Introduction

Rufinamide is an antiepileptic drug approved by the US Food and Drug Administration as an adjunctive treatment of seizures associated with Lennox–Gastaut syndrome in children 4 years and older and adults. Lennox–Gastaut syndrome consists of a variety of treatment-resistant seizures and is most common among pediatric patients [1]. Rufinamide is chemically known as 1-[(2, 6-difluorophenyl) methyl]-1 H-1,2,3-triazole-4 carboxamide (Fig. 1).

The mechanism of action of Rufinamide involves stabilization of the sodium channel inactive state, effectively keeping the ion channels closed. It is believed to prolong the refractory period of voltage-dependent sodium channels, making neurons less likely to fire [2]. To date, all analytical methods described in literature for the determination of Rufinamide in biological fluids involve liquid chromatography [3–7], liquid chromatography–mass spectrometry [8] and HPLC [9] methods. In the present work, we developed a simple, precise, accurate, selective and robust liquid chromatographic method for the determination of Rufinamide in pharmaceutical dosage form as an alternative method.

2. Experimental

2.1. Chemicals and reagents

Rufinamide standard (purity≥98.0%) was obtained from Eisai Pharmaceuticals (Visakhapatnam, India). Acetonitrile (HPLC

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grade), sodium hydroxide, hydrochloric acid and hydrogen peroxide were purchased from Merck (India). Rufinamide is available as tablets with brand names ^{Pr}BANZEL$^{TM®}$ and BANZEL® with label claim of 100, 200 and 400 mg of drug. All chemicals were of analytical grade and used as received.

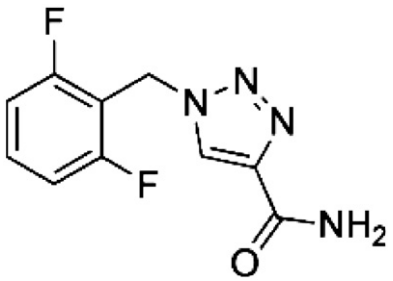

Fig. 1 Chemical structure of Rufinamide.

2.2. HPLC instrumentation and conditions

Chromatographic separation was achieved by using a Shimadzu Model CBM-20 A/20 Alite HPLC system, equipped with an SPD M20A prominence photodiode array detector (250 mm × 4.6 mm, 5 µm particle size) maintained at 25 °C. Isocratic elution was performed using acetonitrile and water (60:40, v/v) with flow rate 0.8 mL/min. 20 µL of sample was injected into the HPLC system.

Rufinamide stock solution (1000 µg/mL) was prepared by accurately weighing 25 mg of Rufinamide in a 25 mL amber volumetric flask and making up to volume with mobile phase. Working solutions for HPLC injections were prepared on a daily basis from the stock solution in a solvent mixture of acetonitrile and water (60:40, v/v) (mobile phase). Solutions were filtered through a 0.45 µm membrane filter prior to injection.

20 tablets from each brand (^{Pr}BANZEL$^{TM®}$ and BANZEL®) were procured, weighed and crushed to a fine powder. Powder equivalent to 25 mg Rufinamide was accurately weighed into a

Table 1 Comparison of the performance characteristics of the present method with the published methods.

S. no.	Method/reagent	λ (nm)	Linearity (µg/mL)	Remarks	Ref.
1.	HPLC (Robotic system)	230	0.05–4.0	Human plasma	[3]
2.	HPLC/acetonitrile: methanol: potassium dihydrogen phosphate	–	0.05–19.09	Plasma (liquid-solid extraction)	[4]
3	HPLC/acetonitrile: methanol: potassium dihydrogen phosphate	–	0.05–20	Plasma and brain	[5]
4	HPLC/acetonitrile: methanol potassium dihydrogen phosphate buffer (pH 4.5)	210	2–40	Very narrow linearity range (UV/visible detector)	[6]
5	HPLC/methanol: dichloromethane:n-hexane	230	0.25–20.0	Plasma and saliva	[7]
6	LC–MS	–	0.48–47.6	Dried blood spots	[8]
7	HPLC/methanol: water (pH 3.0)	220	10–60	Very narrow linearity range	[9]
8	HPLC/acetonitrile: water (60:40, v/v)	215	1.0–200	Wide linearity range stability indicating method (PDA detector)	Present work

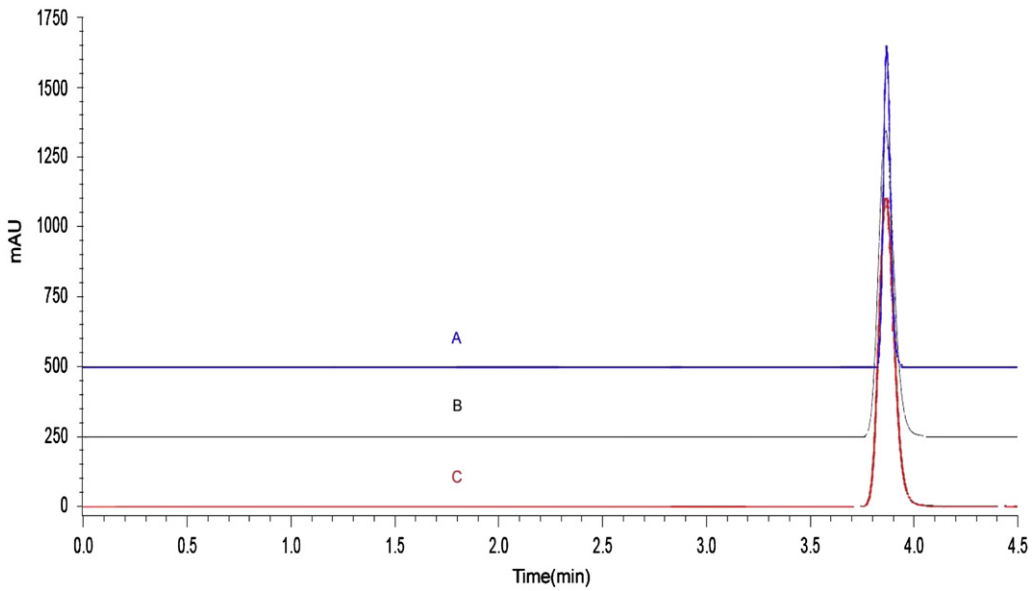

Fig. 2 Representative chromatograms of Rufinamide (50 µg/mL) (A), ^{Pr}BANZEL$^{TM®}$ (400 mg) (B), and BANZEL® (400 mg) (C).

25 mL volumetric flask and made up to volume with mobile phase. The contents of the volumetric flask were sonicated for 30 min to enable complete dissolution of Rufinamide. The solution was filtered and the filtrate was diluted with mobile phase. 20 µL of these solutions were injected into the system and the peak area was recorded from the respective chromatogram.

2.3. Method validation

The method was validated for the following parameters: linearity, precision, accuracy, selectivity, robustness, limit of quantitation (LOQ), limit of detection (LOD) and system suitability [11].

Linearity test solutions for the assay method were prepared from a stock solution at different concentration levels and 20 µL of each solution was injected into the HPLC system and the peak area of the chromatogram obtained was noted.

The intra-day precision of the assay method was evaluated at three concentration levels (10, 20 and 50 µg/mL) ($n=3$) against a qualified reference standard. The inter-day precision study was performed on three different days i.e. day 1, day 2 and day 3 at three different concentration levels (10, 20 and 50 µg/mL) ($n=3$). The %RSD of the obtained assay values at three different concentration levels was calculated.

The accuracy of the assay method was evaluated in triplicate at three concentration levels (80, 100 and 120%), and the percentage recoveries were calculated. The study was carried out in triplicate at 18, 20 and 22 µg/mL.

The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (213 and 217 nm), percentage of acetonitirile in the mobile phase (58 and 62) and flow rate (0.7 and 0.9 mL/min). Robustness of the method was studied using six replicates at a concentration level of 20 µg/mL of Rufinamide.

The LOQ and LOD were based on the standard deviation of the response and the slope of the constructed calibration curve ($n=3$), as described in International Conference on Harmonization guidelines Q2 (R1) [11].

The solutions extracted from the marketed formulations were also injected into the HPLC system and the peak area of the chromatograms was noted. A calibration curve was plotted by taking concentration of the drug solution on the x-axis and the corresponding peak area on the y-axis.

2.4. Forced degradation studies/specificity

The study was intended to ensure the effective separation of Rufinamide and its degradation peaks of formulation ingredients at the retention time of Rufinamide. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method [10].

All solutions for use in stress studies were prepared at an initial concentration of 1 mg/mL of Rufinamide and refluxed for 30 min at 80 °C. All samples were then diluted in mobile phase to give a final concentration of 50 µg/mL and filtered before injection.

Acid decomposition was carried out in 0.1 M HCl and alkaline degradation was conducted using 0.1 M NaOH and refluxed for 30 min at 80 °C. After cooling the solutions were neutralized and diluted with mobile phase.

Solutions for oxidative stress studies were prepared using 3% H_2O_2 at a concentration of 1 mg/mL of Rufinamide and after refluxation for 30 min at 80 °C on the thermostat the sample solution was cooled and diluted accordingly with the mobile phase.

For thermal stress testing, the drug solution (1 mg/mL) was heated in thermostat at 80 °C for 30 min, cooled and used. The drug solution (1 mg/mL) for photo stability testing was exposed to UV light for 4 h UV light chamber (365 nm) and analyzed.

3. Results and discussion

No stability indicating method is available in the official compendia using HPLC for analyzing Rufinamide in dosage forms till now. The present proposed method was compared with the reported methods in the literature and shown in Table 1. The complete separation of the analytes was accomplished in less than 10 min and the method can be successfully applicable to perform long-term and accelerate stability studies of Rufinamide formulations.

Table 2 Linearity of Rufinamide.

Conc. (µg/mL)	*Mean peak area ±SD ($n=3$)	*RSD (%)
1	116922±379	0.32
5	582510±1648	0.28
10	1174190±5836	0.50
20	2262697±7354	0.33
50	5858816±16053	0.27
100	11678667±16584	0.14
150	17018877±55822	0.33
200	22544511±51176	0.23

*Mean of three replicates.

Table 3 Intra-day and inter-day precision studies of Rufinamide.

Sample no.	Conc. (µg/mL)	Intra-day precision		Inter-day precision	
		*Mean peak area ±SD	*RSD (%)	*Mean peak area ±SD	*RSD (%)
1	10	1173321±1678	0.14	1173769±9001	0.76
2	20	2256364±6506	0.29	2264158±13503	0.59
3	50	5846397±13619	0.23	5832216±35964	0.62

*Mean of three replicates.

Initially the stressed samples were analyzed using a mobile phase consisting of water: acetonitrile (70:30, v/v) at a flow rate of 1.0 mL/min. Under these conditions, the resolution and peak symmetry were not satisfactory, so the mobile phase was changed to water: acetonitrile (40:60, v/v) with a flow rate of 0.8 mL/min under which peaks were well resolved with good symmetry and sharpness and therefore mobile phase containing water: acetonitrile (40:60, v/v) was chosen for the entire study.

Table 4 Accuracy–recovery study of Rufinamide by standard-addition method.

Sample no.	Spiked concentration (μg/mL)	*Measured concentration (μg/mL)	Recovery* (%)	*RSD (%)
1	8 (80%)	7.83	97.87	0.71
2	10 (100%)	9.87	98.70	
3	12 (120%)	11.91	99.25	

*Mean of three replicates.

Table 5 Analysis of Rufinamide commercial formulation (tablets).

Sample no.	Formulation	Labeled claim (mg)	*Amount found (mg)	*Recovery (%)
1	^{Pr}BANZEL$^{TM®}$	400	388.92	97.23
2	BANZEL®	400	387.92	96.98

*Mean of three replicates.

Table 6 Forced degradation studies of Rufinamide.

Stress conditions	*Drug recovered (%)	*Drug decomposed (%)
Standard drug	100.00	100.00
Acidic hydrolysis	92.21	7.79
Alkaline hydrolysis	97.16	2.84
Oxidative degradation	94.36	5.63
Thermal degradation	99.96	0.04
Photolytic degradation	99.86	0.14

*Mean of three replicates.

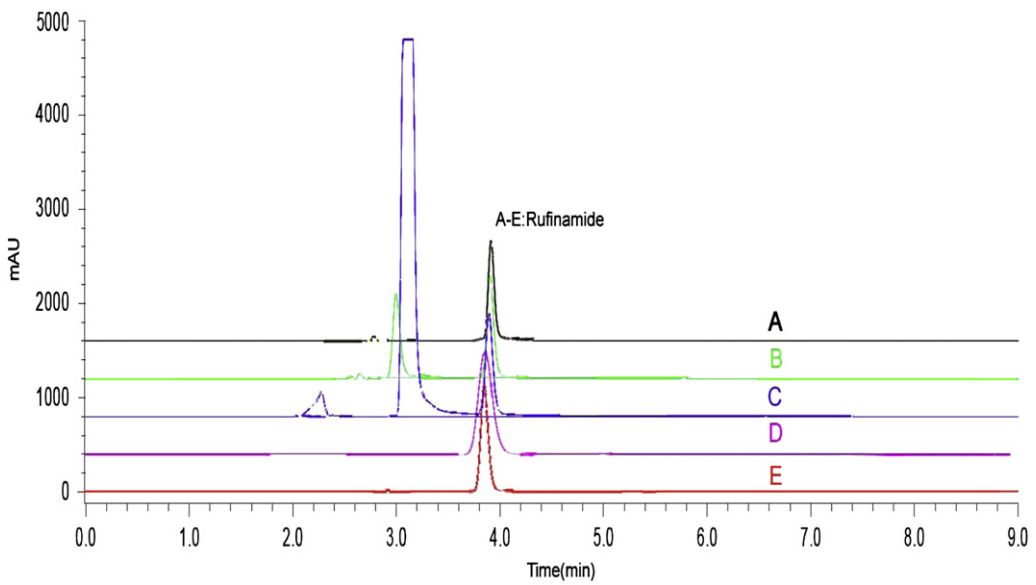

Fig. 3 Representative chromatograms of Rufinamide (50 μg/mL) on acidic (A), alkaline (B), oxidative (C), photolytic (D) and thermal (E) degradations.

The representative chromatogram obtained for Rufinamide is shown in Fig. 2A and those of marketed formulations are shown in Fig. 2B–C. The calibration curve was linear over the concentration range 1–200 μg/mL (Table 2) and the regression equation was found to be $y = 113190\ x + 63053$ with correlation coefficient of 0.9997.

The RSD in precision studies was found to be 0.14–0.29% (Intra-day) and 0.59–0.76% (Inter-day) (Table 3). The % RSD in accuracy studies (Table 4) and robustness studies was found to be less than 2.0%, indicating that the method is precise, accurate and robust. The LOQ was found to be 0.7346 μg/mL and the LOD was found to be 0.2423 μg/mL.

The proposed method was applied for the determination of Rufinamide tablets and the results of these assays yielded 97.23–96.98%, respectively, with RSD < 2.0% (Table 5).

The capacity factor was more than 2, theoretical plates were 8576 (more than 2000) and tailing factor was 1.26 (less than 2) for the Rufinamide peak. The % RSD value of assay determined under original conditions and robustness conditions was less than 2.0%, indicating that the developed method was robust.

During the acidic degradation, 7.79% of the drug was decomposed. The triazole and carboxamide groups present in the Rufinamide chemical structure may be responsible for the reported acidic degradation. During the alkaline degradation a major degradant was observed at 2.987 mins without interfering the elution of drug peak (3.891 mins) and the percentage of drug decomposition was found to be 2.84%. Rufinamide has undergone thermal, oxidation and UV degradation slightly i.e less than 6.0% (Table 6). Typical chromatograms obtained following the assay of stressed samples are shown in Fig. 3.

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