

Development and Validation of Chromatographic and Spectrophotometric Methods for the Quantitation of Rufinamide in Pharmaceutical Preparations

Short title: Chromatographic and spectrophotometric Methods for Rufinamide

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ABSTRACT

INTRODUCTION: Objectives: Two optimized and validated HPLC and spectrophotometric methods were proposed. The developed methods were quantified with high sensitivity, accuracy, and precision at low concentrations to determine rufinamide in active pharmaceutical ingredient (API) and pharmaceutical preparations.

METHODS: Materials and Methods: HPLC method was developed using BDS Hypersil C18 column and combination of methanol: acetonitrile: water (15: 10: 75, v/v/v) as mobile phase and detected at 210 nm. A reaction of rufinamide with sodium nitrite and hydrochloric acid took place, absorbed maximally at 385 nm was extended to develop a UV-visible spectrophotometric method to determine rufinamide in API and pharmaceutical preparations.

RESULTS: Results: Different analytical validation parameters include specificity, linearity, accuracy, precision, the limit of detection, quantification, ruggedness, and robustness, were determined as per International Conference on Harmonisation (ICH) guidelines. The linearity range of rufinamide was 0.15-3.5 and 10-100 µg/mL for HPLC and spectrophotometric method, respectively.

DISCUSSION AND CONCLUSION: Conclusion: The statistical analyses disclosed no significant differences between the acquired results and reported ones. The proposed investigations were valuable for drug monitoring and regular analysis of rufinamide in quality control and research laboratories. Moreover, the accuracy and precision obtained with the UV-Visible spectrophotometry implied that it could be a cheap, easy, and alternative method, while the HPLC could be a sensitive one to determine rufinamide with low concentration levels.

Keywords: Rufinamide, validation, quality control laboratories, HPLC, UV-Visible spectrophotometry

INTRODUCTION

Rufinamide (RUF) is a third-generation antiepileptic drug used to treat a neurological disorder characterised by seizure symptoms linked with Lennox-Gastaut syndrome (LGS). LGS is rare and one of the most severe forms of epilepsy among children between typically 3 to 5 years and adults. Therefore, the treatment of LGS is highly important, especially in childhood epilepsy. However, treatment success is limited in this condition.^{1,2} RUF is a triazole derivative classified as an orphan drug, chemically known as 1-[(2,6-difluorophenyl) methyl]-1H-1,2,3-triazole-4-carboxamide (mol. formula: C₁₀H₈F₂N₄O, M. Wt 238.2 g/mol) developed in 2004 and has been authorised by the US Food and Drug Administration (US FDA) in 2008 for the management of seizure associated with LGS³⁻⁸ in children (four years and above) and adults. RUF is believed to increase the refractory period of voltage-dependent sodium channels, reducing the possibility of fire in neurons.⁹ The carboxamide group of RUF is extensively metabolised via carboxylesterase-mediated hydrolysis in a pharmacologically inactive carboxylic acid derivative and finally excreted in the urine. It has been recommended to monitor the absorption of this drug (slow and dose-dependent), as its peculiar and probable interaction with co-administered antiepileptic agents leads to pharmacokinetic variability. Therefore, regular therapeutic drug monitoring must adjust optimal dosage according to the patient's individual needs with epileptic seizures.¹⁰ Chromatographic methods with different detection techniques such as HPLC/UV and LC-MS/MS.¹¹⁻²⁵ has been developed. Recently, stability-indicating RP-HPLC and first derivative ratio assays were designed to determine RUF in the presence of an alkaline degradation product in dosage forms.²⁶ A validated high-performance thin-layer chromatographic (HPTLC) assay in bulk drug and its formulations were also developed.²⁷ Only a few low sensitive spectrophotometric methods in pharmaceutical dosage forms, human and animal biological fluids,²⁸⁻³⁰ and extraction-based spectrophotometric methods were developed to determine RUF.³¹⁻³²

The majority of the reported methods have several drawbacks and are not stability-indicating. Hence, there is a need to develop sensitive, validated and simple analytical methodologies such as HPLC and UV-Visible spectrophotometry and estimate the accurate and precise drug content in pharmaceutical preparations. HPLC and UV-Visible spectrophotometric techniques are widely employed in pharmaceutical quality control laboratories to quantify drug substances. The HPLC method is characterised by sensitivity, repeatability, specificity, and spectrophotometric techniques, which can be considered inexpensive, simple, fast, and direct.

This research report aimed to develop two well-optimized and validated analytical methods (HPLC and Spectrophotometry) with high sensitivity, accuracy, and precision with a good linearity range for RUF determination in pure and pharmaceutical preparations.

MATERIALS AND METHODS

Products and reagents

Sodium nitrite (NaNO₂), hydrochloric acid (HCl), methanol (CH₃OH), dimethylformamide [(CH₃)₂NCH], and acetonitrile (CH₃CN) were purchased from Sigma Aldrich through a local vendor. All the reagents are analytical grade and utilised without additional purification. The pharmaceutical product, Banzel 200 and 400 mg, belongs to Eisai Co. Ltd.

Instrumentation and analytical conditions

Shimadzu, LC–2010 CHT HPLC was utilised to separate for the separation. HPLC system consists of a pump (LC–20AD), autosampler (SIL–20AC), column oven (CTO–20AC), and photodiode array detector (SPD–20A). LC solution software was used to integrate the chromatograms. The column used to separate the analytes was BDS Hypersil C₁₈ (250 mm × 4.6 mm, 5 μm). The column temperature was maintained at 30° C with a mobile phase comprised of methanol: acetonitrile: dimethylformamide (7:5:8, v/v/v) with a fixed flow rate (1 mL/min). An injection volume of 10 μL was chosen and detected at 210 nm. All the spectral runs were performed with Jenway (UV–Vis 6300) and Cecil (CE 7400) spectrophotometers with 10 mm path length at wavelength 385 nm.

Extraction of Rufinamide from the dosage forms

Five RUF tablets (200 mg/tablet) are ground into powder, shifted into a 1000 mL beaker, and dissolved in dimethylformamide and distilled water (1:10). Column chromatography utilised, stationary phase as silica gel, and the mobile phase combines methanol: water: glacial acetic acid (6.3: 1.3: 0.5 v/v/v), separated and dried as solid RUF.

Preparation of standard solutions

For the HPLC method, the RUF stock solution (50 μg/mL) was prepared in a 100 mL volumetric flask by transferring a correct amount of the drug in dimethylformamide (DMF). Then, sonicated the mixture for 15 minutes, and finally, the volume was completed with the DMF. This solution was further diluted as per the requirement for the analysis.

A stock solution of RUF (1 mg/mL) was prepared for the spectrophotometric method, prepared in DMF. The HCl (0.50M) and NaNO₂ (0.10M) were diluted and prepared with distilled water, and further dilutions continued as necessary.

Optimization of Variables

The trial of the current HPLC procedure was performed with several columns such as ODS Hypersil C₁₈ (250 mm × 4.6 mm, 5 μm), ODS Hypersil C₁₈ (150 mm × 4.6 mm, 5 μm), ODS Hypersil C₈ (250 mm × 4.6 mm, 5 μm), ODS Hypersil C₈ (150 mm × 4.6 mm, 5 μm), BDS Hypersil C₁₈ (250 mm × 4.6 mm, 5 μm), BDS Hypersil C₁₈ (150 mm × 4.6 mm, 5 μm), BDS Hypersil C₈ (250 mm × 4.6 mm, 5 μm) and BDS Hypersil C₈ (150 mm × 4.6 mm, 5 μm). The best separation was achieved with BDS Hypersil C₈ (250 mm × 4.6 mm, 5 μm). Different solvents with ratio, as mobile phase studied and the highest separation occurs with methanol: acetonitrile: dimethylformamide (7:5:8, v/v/v) at controlled oven temperature 30°C with detection at 210 nm. The effect of the volume of 0.50 M HCl concentration was studied using spectrophotometry by keeping the constant concentration of RUF (100 μg/mL) and 1 mL NaNO₂ (0.10M) with a varied concentration of HCl (0.1–1.1 mL) in a final volume of 10 mL solution. Similarly, the influence of 0.10M NaNO₂ solution concentration was also studied by keeping the constant concentrations of 1 mL RUF (100 μg/mL) and the optimized concentration of 0.50M HCl (0.9 mL) and varying the concentration of NaNO₂ (0.1–2.4 mL) in a final volume of 10 mL solution. Figure 1 shows an increase in the absorbance of 0.5M HCl concentration up to 0.7 mL and the influence of 0.10M NaNO₂ solution concentration on the absorbance up to 1.8 mL. Therefore, a concentration of 0.9 mL of 0.50M HCl and 2.1 mL of 0.1M NaNO₂ was used throughout the experiment. The figure also includes the error bar with respective standard deviations for optimizing HCl and NaNO₂.

Analytical Method Validation

The optimized spectrophotometric method was validated by evaluating linearity, accuracy, precision, the limit of detection (LOD), the limit of quantitation (LOQ), specificity, standard addition, ruggedness and robustness following the ICH guideline Q2 (R1).³³

Linearity

Aliquots of 0.1–1.0 mL from 100 µg/mL RUF were pipetted into a series of 10 mL standard volumetric flasks. To each flask, 0.9 mL of 0.50 M HCl, followed by 2.1 mL of 0.10 M sodium nitrite, were added and completed the volume with doubly distilled water. The contents of each flask mixed well and heated at 100°C, and the increase in absorbance was recorded immediately at 385 nm.

Into a sequence of 10 volumetric flasks with a 50 mL capacity, different RUF (50 µg/mL) volume was transferred to prepare in the range of 0.1–4.5 µg/mL. 10 µL of each one was injected five replicate times, and the average peak area was recorded to evaluate the developed method's linearity range.

Limit of detection and Limit of quantitation

Both methods (Spectrophotometric and HPLC) sensitivity were established with the limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ value were computed with the help of a calibration curve, following the below equations:

$$\text{LOD} = 3.3 \times S_0/m, \text{ and } \text{LOQ} = 10 \times S_0/m,$$

where S_0 = standard deviation of the y-intercept of a regression line

m = slope of the calibration curve

Accuracy and Precision

HPLC and spectrophotometric method's accuracy and precision were assessed. It determines the drug concentration at three different concentration levels (low, medium and high) within one day (intraday) and consecutive five days (interday). The standard deviation (SD), percent relative standard deviation (%RSD) was determined. The standard addition method continued to obtain the percent recoveries.

Robustness

For the assessment of method robustness, slight variation was considered with the current experimental parameters. The analysis was presented at the deliberately varied experimental conditions by taking two different wavelengths (± 2 nm) and mobile phase composition ratio. The SD and %RSD was calculated.

Ruggedness

Small changes in the environment conducted experiments, and a model of instrument mean little variation with operating conditions than the standard proposed method of analysis.

RESULTS AND DISCUSSION

RUF, an US FDA approved drug is a triazole derivative structurally unrelated to other marketed antiepileptic drugs. It is highly susceptible to acidic and alkaline hydrolysis. At the same time, it remained stable under oxidative, thermal, and photolytic stress conditions.³⁴ Literature reported that RUF could extensively metabolise after the hydrolysis of the carboxamide group of the drug via a primary biotransformation pathway (carboxylesterases) into an inactive acid derivative that is eliminated mainly in the urine.^{35,36} Based on the above facts, a reaction of RUF with NaNO_2 and HCl performed at 100°C undergoes hydrolysis of the carboxamide group of the drug and is expected to convert into a yellow-coloured acid derivative which absorbs maximally at 385 nm. A scheme was proposed based on a literature survey (Figure 2).

Under optimized chromatographic conditions, the RUF separated with a higher number of theoretical plates, good resolution and peak shape. There will be no interference from other components with a retention time of 4.65 minutes (Figure 3).

The specificity/system suitability test runs to ensure the current procedures connect all the requirements to start the analysis. Generally, it determines in the presence of common excipients available with the pharmaceutical dosage form to know the methods' ability to separate without interference. The %RSD was calculated for both practices and found to be less than 2%.

Under the optimized experimental conditions described, Beer's law obeyed over the concentration ranges of 10–100 µg/mL for the spectrophotometric method. The linear regression analysis using the least square method was made to assess slope, intercept, and regression coefficient. High values of the regression coefficient and the small values of the intercept of the regression equation proved the linearity of the calibration curve. The values of the limit of detection and limit of quantitation reveal the high sensitivity of the proposed methods (Table 1). The HPLC procedure was rectilinear within the range of 0.15–3.5 µg/mL. The results proved an outstanding correlation between the peak area and each drug's concentration within the specified range (Table 1).

The LOD and LOQ are the smallest concentration that provides a noticeable response and possibly to be quantified. Consequently, computed the signal to noise (S/N) ratio. Then calculated the LOD, LOQ value 0.061, 4.07 and 0.184, 12.33 µg/mL, of the current methods, respectively. The replicate analysis (n=5) of RUF corresponding to 1, 2, 3 and 20, 60, 100 µg/mL of the proposed HPLC and UV–Visible spectrophotometric methods, were performed, respectively to determine its interday and interday precision. The %RSD were in the ranges 0.101–0.637% and 0.302–0.807, respectively, for spectrophotometric and HPLC methods (Table 2). The accuracy parameter was determined with the help of the standard addition method. Due to that, 50, 100, and 150 % spiked with the original drug components and determined its %recovery. The computed value was in the range of 98–100 % for both methods (Table 3).

The robustness of the method relative to each functioning parameter was studied and verified. The impacts of variation with wavelength (± 2) and mobile phase composition ($\pm 2\%$) were analysed to determine the method's robustness. The % recovery and RSD were in the range of 99.15–99.56 and 0.123–0.612 % for both methods.

The ruggedness studies were performed with a different model of instrument. The % recovery \pm RSD resulted within the range of 98–102 and $\pm 2\%$, as per the ICH guidelines.³³ All results were reproducible and indicated the proposed methods are robust to determine the RUF in pharmaceuticals.

CONCLUSION

HPLC and UV-Visible spectrophotometric methods were found to be appropriate methods to quantify rufinamide in pure and pharmaceutical preparations. Therefore, precise and selective HPLC and spectrophotometric methods were developed to estimate rufinamide in pharmaceutical preparations. Although HPLC is a modern and sophisticated technique, however, it is expensive and time-consuming. A narrow range of rufinamide concentrations (0.15–3.5 µg/mL) could be estimated using HPLC. On the other hand, the UV-Visible spectrophotometric method is easy,

inexpensive, found almost in all quality control and research laboratories, and determined a wide range of RUF concentrations (10-100 µg/mL). The chromatographic method presented sensitive, reliable results with good recoveries. In contrast, the spectrophotometric method offered a simple, accurate, precise, and time-saving method and could be recommended as an equivalent alternative method. These two methods could be successfully applied to quantitate RUF in research laboratories, hospitals, and quality control laboratories.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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Table 1. Summary of the linearity data for the spectrophotometry and HPLC methods

Parameter	UV-Visible Spectrophotometry	HPLC
Beer's law range ($\mu\text{g/mL}$)	10-100	0.15-3.5
Regression equation	$y=0.0078x - 0.0059$	$y=2332.2x + 970.72$
S_0	0.009697	42.82088
M (Slope)	0.007863	2332.155
Regression coefficient (r^2)	0.9984	0.9998
LOD ($\mu\text{g/mL}$)	4.07	0.061
LOQ ($\mu\text{g/mL}$)	12.33	0.184

Table 2. Determination of RUF in pharmaceutical formulation for precision

Proposed methods	Amount ($\mu\text{g/mL}$)		% Recovery	% RSD ^a	SAE ^b	CL ^c	
	Taken	Found \pm SD ^a					
UV-Visible Spectrophotometry	Intraday	20	19.67 \pm 0.112	98.35	0.569	0.050	0.139
		60	59.92 \pm 0.154	99.87	0.257	0.069	0.191
		100	99.81 \pm 0.101	99.81	0.101	0.045	0.125
	Interday	20	19.63 \pm 0.125	98.15	0.637	0.056	0.155
		60	59.13 \pm 0.177	98.55	0.299	0.079	0.219
		100	99.56 \pm 0.131	99.56	0.132	0.059	0.163
HPLC	Intraday	1	0.989 \pm 0.005	98.90	0.506	0.002	0.006
		2	1.987 \pm 0.006	99.35	0.302	0.003	0.008
		3	2.963 \pm 0.009	98.77	0.304	0.004	0.011
	Interday	1	0.992 \pm 0.008	99.20	0.807	0.004	0.010
		2	1.976 \pm 0.011	98.80	0.557	0.005	0.014
		3	2.983 \pm 0.014	99.43	0.469	0.006	0.017

Mean for 5 independent analyses. ^aSD, standard deviation, RSD, relative standard deviation; ^bSAE, standard analytical error; ^cC.L., confidence limit at 95 % confidence level and 4 degrees of freedom ($t=2.776$).

Table 3. Standard addition method to determine the accuracy of the proposed method

Proposed methods		Amount ($\mu\text{g/mL}$)			% Recovery	% RSD ^a	SAE ^b	CL ^c
		Taken	Added	Found \pm SD ^a				
UV-Visible Spectrophotometry	Intraday	20	20	39.75 \pm 0.145	99.38	0.365	0.065	0.180
		20	40	59.92 \pm 0.123	99.87	0.205	0.055	0.153
		20	60	78.99 \pm 0.132	98.74	0.167	0.059	0.164
	Interday	20	20	39.88 \pm 0.167	99.70	0.419	0.075	0.207
		20	40	59.64 \pm 0.153	99.40	0.257	0.068	0.190
		20	60	79.11 \pm 0.148	98.87	0.187	0.066	0.184
HPLC	Intraday	0.8	0.8	1.58 \pm 0.003	98.75	0.190	0.001	0.004
		0.8	1.6	2.39 \pm 0.004	99.58	0.167	0.002	0.005
		0.8	2.4	3.17 \pm 0.007	99.06	0.221	0.003	0.009
	Interday	0.8	0.8	1.57 \pm 0.006	98.13	0.382	0.003	0.007
		0.8	1.6	2.38 \pm 0.009	99.17	0.378	0.004	0.011
		0.8	2.4	3.19 \pm 0.005	99.69	0.157	0.002	0.006

Mean for 5 independent analyses. ^aSD, standard deviation, RSD, relative standard deviation; ^bSAE, standard analytical error;

°C.L., confidence limit at 95 % confidence level and 4 degrees of freedom (t=2.776).

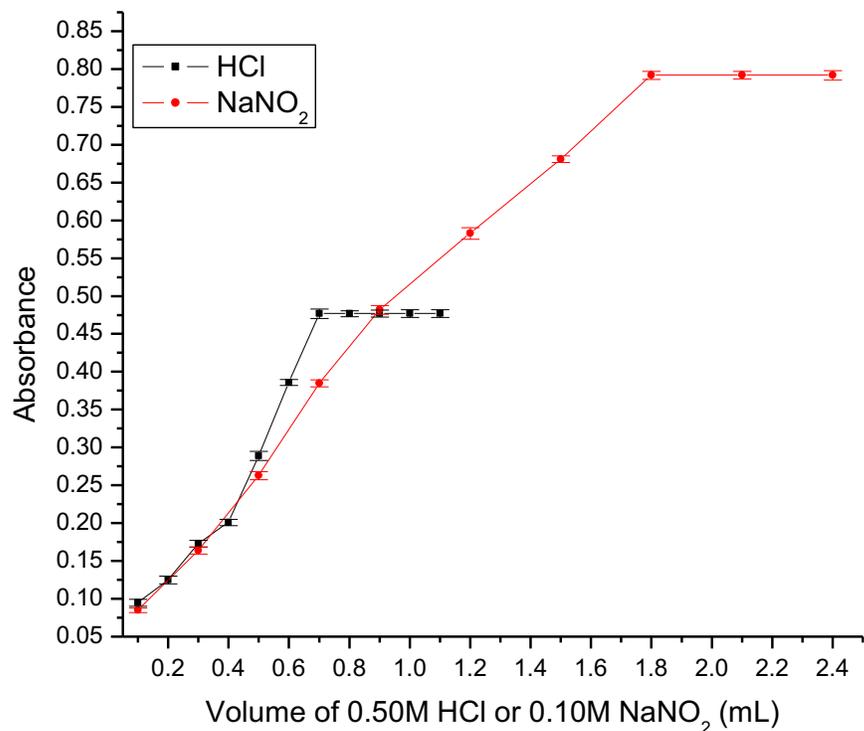


Figure 1. Effect of concentration and error bar with standard deviations of HCl/ NaNO₂

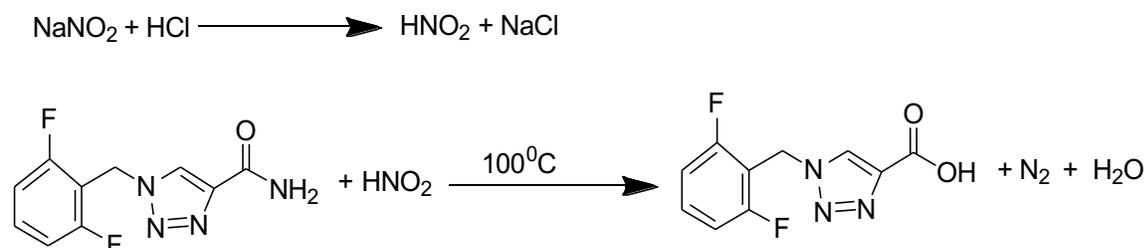


Figure 2. Proposed reaction scheme

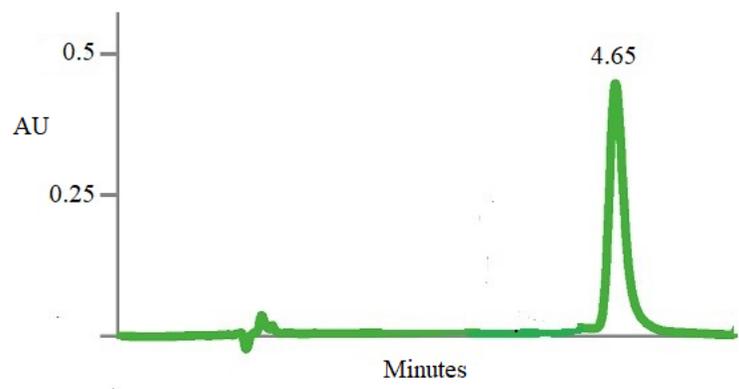


Figure 3. RUF chromatogram with retention time 4.65 minutes