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ORIGINAL ARTICLE

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

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ABSTRACT

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

Materials and Methods: HPLC method was developed using a base deactivated silica Hypersil C_{18} column and a combination of methanol: acetonitrile: water (15: 10: 75, v/v/v) as the mobile phase and detected at 210 nm. A reaction of RUF with sodium nitrite and hydrochloric acid occurred, absorbed maximally at 385 nm was extended to develop a ultraviolet (UV)-visible spectrophotometric method to determine RUF in API and pharmaceutical preparations.

Results: Different analytical validation parameters, including specificity, linearity, accuracy, precision, the limit of detection, quantification, ruggedness, and robustness, were determined as *per* International Conference on Harmonization guidelines. The linearity range of RUF was 0.15-3.5 and 10-100 µg/mL for HPLC and spectrophotometric methods, respectively.

Conclusion: The proposed investigations were valuable for drug monitoring and regular analysis of RUF in quality control and research laboratories. Moreover, the accuracy and precision obtained with the UV-visible spectrophotometer implied that it could be a cheap, easy, and alternative method, while HPLC could be sensitive to determine RUF at low concentration levels.

Key words: Rufinamide, validation, quality control laboratories, HPLC, UV-visible spectrophotometry

INTRODUCTION

Rufinamide (RUF) is a third-generation antiepileptic drug used to treat a neurological disorder characterized by seizure symptoms linked to 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 treatmeant of LGS is highly important, particularly in patients with childhood epilepsy. However, treatment success is limited by 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: $C_{10}H_8F_2N_4O$, MW: 238.2 g/mol) developed in 2004 and has been authorized by the US Food and Drug Administration (US FDA) in 2008 for managing seizures associated with LGS[3-8] in children (4 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 metabolized *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

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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 high performance liquid chromatography/ultraviolet (HPLC/UV) and liquid chromatography-tandem mass spectrometry[11-25] (LC-MS) have been developed. Recently, stability-indicating reversed phase-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 (TLC) 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 have been developed to determine RUF.[31,32]

Most 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, which are widely employed in pharmaceutical quality control laboratories to quantify drug substances, and to estimate accurate and precise drug content in pharmaceutical preparations. HPLC method is characterized by sensitivity, repeatability, specificity, and spectrophotometric techniques, considered inexpensive, simple, fast, and direct.

This research 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 ($NaNO_2$), hydrochloric acid (HCl), methanol (CH_3OH), dimethylformamide [$(CH_3)_2NCH$], and acetonitrile (CH_3CN) were purchased from Sigma Aldrich through a local vendor. All the reagents are analytical grade and can be utilized without additional purification. Banzel 200 and 400 mg of the pharmaceutical product belonged to Eisai Co. Ltd.

Instrumentation and analytical conditions

Shimadzu, LC-2010 CHT HPLC was used to separate, which 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 base-deactivated silica (BDS) Hypersil C_{18} (250 mm × 4.6 mm, 5 µm). The column temperature was maintained at 30°C with a mobile phase comprised 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 using Jenway (UV-vis 6300) and cecil (CE-7400) spectrophotometers with 10 mm path length at a wavelength of 385 nm.

Extraction of RUF from the dosage forms

Five RUF tablets (200 mg/tablet) were ground into powder, shifted into a 1000 mL beaker and dissolved in dimethylformamide and distilled water (1: 10). Stationary phase used in column chromatography was silica gel. Mobile phase consisted of methanol: water: glacial acetic acid (6.3: 1.3: 0.5 v/v/v), separated and dried as a solid RUF.

Preparation of standard solutions

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

A stock of RUF (1 mg/mL) was prepared for the spectrophotometric method in DMF. The HCl (0.50 M) and $NaNO_2$ (0.10 M) were diluted and prepared with distilled water, and further dilutions were continued as necessary.

Optimization of variables

Trial of current HPLC procedure was performed using several columns such as ODS Hypersil C_{18} (250 mm × 4.6 mm, 5 µm), ODS Hypersil C_{18} (150 mm × 4.6 mm, 5 µm), ODS Hypersil C_8 (250 mm × 4.6 mm, 5 µm), ODS Hypersil C_8 (150 mm × 4.6 mm, 5 µm), BDS Hypersil C_{18} (250 mm × 4.6 mm, 5 µm), BDS Hypersil C_{18} (150 mm × 4.6 mm, 5 µm), BDS Hypersil C_8 (250 mm × 4.6 mm, 5 µm), and BDS Hypersil C_8 (150 mm × 4.6 mm, 5 µm). The best separation was achieved with BDS Hypersil C_8 (250 mm × 4.6 mm, 5 µm). Different solvents with ratio, as the mobile phase, were studied and the highest separation occurs with methanol: acetonitrile: dimethylformamide (7: 5: 8, v/v/v) at a controlled oven temperature 30°C with detection at 210 nm.

Effect of volume of 0.50 M HCl concentration was studied using spectrophotometry by keeping a constant concentration of RUF (100 µg/mL) and 1 mL $NaNO_2$ (0.10 M) with a varied concentration of HCl (0.1-1.1 mL) in a final volume of 10 mL solution. Similarly, influence of 0.10 M $NaNO_2$ solution concentration was also studied by keeping the constant concentrations of RUF (100 µg/mL) and the optimized concentration of 0.50 M HCl (0.9 mL) and varying the concentration of $NaNO_2$ (0.1-2.4 mL) in a final volume of 10 mL solution. Figure 1 shows an increase in the absorbance of 0.5 M HCl concentration up to 0.7 mL and the influence of 0.10 M $NaNO_2$ solution concentration on the absorbance up to 1.8 mL. Therefore, concentrations of 0.9 mL of 0.50 M HCl and 2.1 mL of 0.1 M $NaNO_2$ were used throughout the experiment. The figure also includes an error bar with the respective standard deviations for optimizing HCl and $NaNO_2$.

Analytical method validation

The optimized spectrophotometric method was validated by evaluating the linearity, accuracy, precision, the limit of detection (LOD), the limit of quantitation (LOQ), specificity, standard addition, ruggedness, and robustness following the International Conference on Harmonization (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 was added, followed by 2.1 mL of 0.10 M $NaNO_2$. The volume was completed with double distilled water. The contents of each flask were mixed well and heated at 100˚C. The increase in absorbance was recorded immediately at 385 nm.

Into a sequence of ten volumetric flasks with a 50 mL capacity, different RUF (50 µg/mL) volumes were transferred to prepare in the range of 0.15-3.5 µg/mL. 10 µL of each one was injected in 5 replicates and average peak area was recorded to evaluate the developed method's linearity range.

LOD and LOQ

Both methods (spectrophotometric and HPLC) sensitivities were established with the LOD and the LOQ. The LOD and LOQ values were computed with the help of a calibration curve, following the equations given below:

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

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 precision were assessed. It determines the drug concentration at three different concentration levels (low, medium and high) within one day (intraday) and 5 consecutive days (interday). The standard deviation (SD) and percentage relative SD (RSD%) were determined. The standard addition method was continued to obtain percentage recoveries.

Robustness

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

Ruggedness

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

Statistical analysis

Detailed statistical data analyses are presented in Table 1 for the proposed methods. The results proved an outstanding correlation between peak area and each drug's concentration within the specified range.

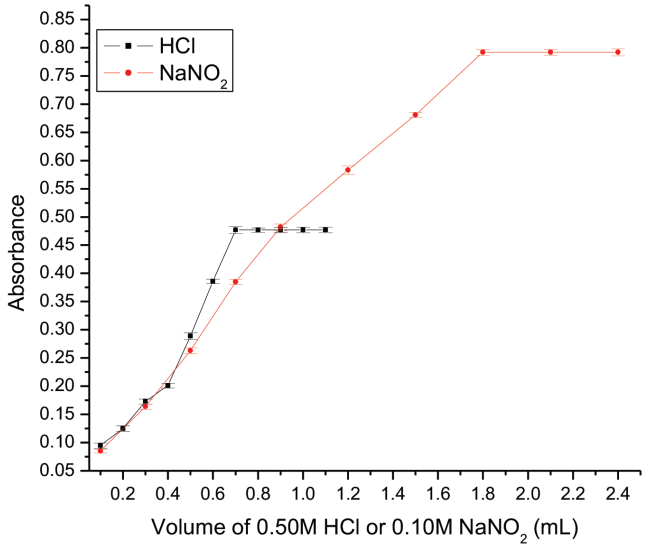

Figure 1. Effect of concentration and error bars with standard deviations of HCl/ $NaNO_2$

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. Simultaneously, it remained stable under oxidative, thermal, and photolytic stress conditions.[34] Literature reported that RUF could extensively metabolize 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 that absorbs maximally at 385 nm. A scheme was proposed based on a literature survey (Figure 2).

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

The specificity/system suitability test runs to ensure the current procedures connect all the requirements to start the analysis. Generally, it determines the presence of common excipients available with the pharmaceutical dosage form to know the

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

Parameter	UV-visible spectrophotometry	HPLC
Beer's law range (µg/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 (µg/mL)	4.07	0.061
LOQ (µg/mL)	12.33	0.184

LOD: Limit of detection, LOQ: Limit of quantitation

methods' ability to separate without interference. RSD% was calculated for both practices and found to be less than 2%.

Under optimized experimental conditions described, Beer's law obeyed the concentration ranges of 10-100 µg/mL for spectrophotometric method. The linear regression analysis used the least square method to assess the slope, intercept, and regression coefficient. High values of the regression coefficient and the small values of the regression equation intercept proved the calibration curve's linearity. The detection and quantification limit values reveal the proposed methods' high sensitivity. The HPLC procedure was rectilinear within 0.15-3.5 µg/mL.

LOD and LOQ are the smallest concentrations that provide a noticeable response and possibly be quantified. Consequently, signal to noise ratio was computed. Then, the current methods calculated the LOD and LOQ values of 0.061, 4.07, and 0.184, 12.33 µg/mL, respectively. The replicated analysis (n= 5) of RUF corresponding to 1, 2, and 3, as well as 20, 60, and 100 µg/mL of the proposed HPLC and UV-visible spectrophotometric methods were performed, determining its intraday and interday

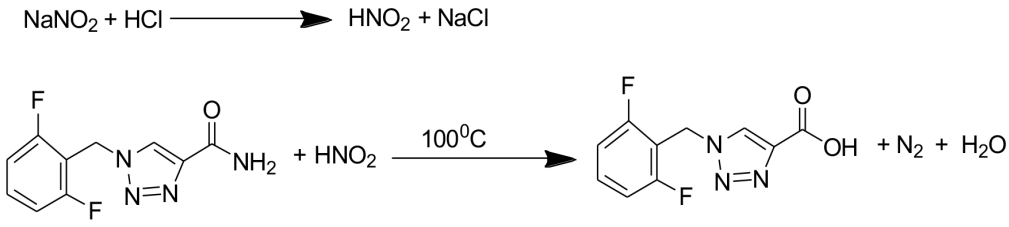

Figure 2. Proposed reaction scheme

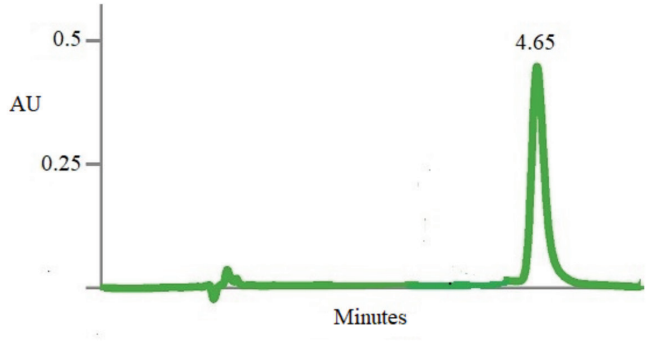

Figure 3. RUF chromatogram with a retention time of 4.65 min

Table 2. Determination of RUF in pharmaceutical formulations for precision

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

Mean for 5 independent analyses. [a]SD: Standard deviation, RSD: Relative standard deviation, [b]SAE: Standard analytical error, [c]CL: Confidence limit at 95% confidence level and 4 degrees of freedom (t= 2.776)

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

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

Mean for 5 independent analyses. [a]SD: Standard deviation, RSD: Relative standard deviation, [b]SAE: Standard analytical error, [c]CL: Confidence limit at 95% confidence level and 4 degrees of freedom (t= 2.776)

precision. For spectrophotometric and HPLC methods, the % RSD was 0.101-0.637% and 0.302-0.807, respectively (Table 2). The accuracy parameter was determined with help of the standard addition method. Due to that, 50, 100, and 150% were spiked with the original drug components and determined its % recovery. The computed value was 98-100% for both methods (Table 3).

The method's robustness relative to each functioning parameter was studied and verified. The impacts of variation with wavelength (± 2) and mobile phase composition (± 2%) were analyzed to determine the method's robustness. Recovery% and RSD were 99.15-99.56 and 0.123-0.612% for both methods.

Ruggedness studies were conducted with a different model of instrument. As *per* ICH guidelines, recovery% ± RSD resulted within 98-102 and ± 2%.[33] All results were reproducible and indicated that the proposed methods are robust enough to determine the RUF in pharmaceuticals.

CONCLUSION

HPLC and UV-visible spectrophotometric methods were appropriate to quantify RUF in pure and pharmaceutical preparations. Therefore, precise and selective HPLC and spectrophotometric methods were developed to estimate RUF in pharmaceutical preparations. Although HPLC is a modern and sophisticated technique, it is expensive and time-consuming. A narrow range of RUF concentrations (0.15-3.5 µg/mL) could be estimated using HPLC. However, the UV-visible spectrophotometric method is easy, inexpensive and performed almost in all quality control and research laboratories. It can also determine various RUF concentrations (10-100 µg/mL). The chromatographic method presented sensitive and reliable results with good recoveries. In contrast, the spectrophotometric method offers a simple, accurate, precise, and time-saving method. It could be recommended as an equivalent alternative method. These two methods could be successfully applied to quantify RUF in research laboratories, hospitals, and quality control laboratories.

Ethics

Ethics Committee Approval: The ethics committee approval is not required for the proposed research. We have not used any human beings or animal matrix.

Informed Consent: Not applicable.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: H.R., S.M.H., Design: S.M.H., Data Collection or Processing: H.R., Analysis, or Interpretation: H.R., S.M.H., Literature Search: H.R., Writing: H.R., S.M.H.

Conflict of Interest: No conflict of interest was declared by the authors.

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