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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR THE ESTIMATION OF NOVEL ANTICANCER DRUG REGORAFENIB MONOHYDRATE IN BULK AND MARKETED FORMULATIONS

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ABSTRACT

The aim of the study was to develop and validate a novel anticancer drug regorafenib monohydrate (RGF MH) for estimation in pure form and in commercially availble marketed tablets. A simple, accurate, precise and rapid UV Spectrophotometric method was developed using methanolic HCl (solvent A) and acetonitrile:methanol (solvent B). The developed method was extensively validated in terms of linearity, accuracy, precision, specificity, limit of detection and limit of quantification according to ICH Q2 (R1) guidelines. Statistical validation included recovery studies for accuracy, linearity, precision, repeatability, and reproducibility. The absorption maxima at 261 nm and 263 nm for solvent A and solvent B respectively, demonstrated linearity within the range of 1-14 µg/ml, with robust correlation coefficients (R^2 = 0.9998 and 0.9999) and recovery rates between 100 to 101.1%. The P-value (< 0.0001) exhibited the statistical significance of the proposed method. Both solvents A and B demonstrated precision and reproducibility, with relative standard deviation values (< 1). Stability studies indicated that the drug remained stable within acceptable limits over a period of 24 h. The developed approach was applied to commercially available tablets of RGF MH, resulting in outcomes consistent with the labeled claims. The percentage recovery demonstrated the lack of interference from excipients present in the formulations. The proposed method for estimating RGF MH has proven to be specific, simple, precise, rapid, and accurate. Hence, developed method is suitable for monitoring RGF MH drug content in

routine analysis within quality control laboratories, applicable to both pure and commercially available dosage forms.

KEYWORDS: UV Spectrophotometer, regorafenib monohydrate, validation, accuracy, precision, ICH.

INTRODUCTION

Pharmaceutical analysis is a fundamental practice which plays an important role in examining both pharmaceutical formulations and bulk drugs, contributing significantly to quality control and assurance. The outcome data generated through analytical test methods serve as a crucial foundation for quality control laboratories, ensuring the identity, purity, potency, and performance of drugs. This information plays a pivotal role in the discovery, development, and manufacturing processes of pharmaceuticals.[1,2] The primary objective of the present study was to establish a rapid UV spectroscopic method for estimating the novel anticancer drug, Regorafenib Monohydrate (RGF MH), in both bulk and marketed formulations, aligning with the validation guidelines set by the Food and Drug Administration (FDA). RGF MH is an orally administered small molecule, non-biologic anticancer drug (refer to Figure 1), specifically targeting vascular endothelial growth factor (VEGF) receptors in stromal cells with angiogenic and oncogenic activities. Recently approved by the US FDA in 2012, as well as by the Japan and European Medicines Agency (EMA) in 2013[3-6], it is employed in the treatment of metastatic colorectal cancers and advanced gastrointestinal tumors (GIST). The model drug is classified as a Class II compound in the Biopharmaceutics Classification System (BCS), and exhibits low solubility and high permeability.[7]

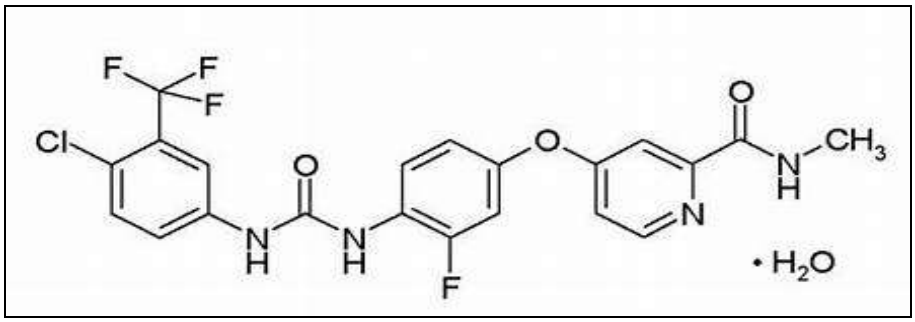

Figure 1: Chemical structure of Regorafenib monohydrate.

Through an extensive literature search, it was found that only a limited number of researchers have undertaken the task of developing and validating a method for the analysis of pure RGF MH. And very few researchers made an effort for the method development and validation for

the pure RGF MH and its metabolites in mouse plasma by a ultra-performance liquid chromatography (UPLC) method and mass spectrometric assay since the drug undergoes extensive hepatic metabolism.[8,9] Further, Kim JS et al[10] established a HPLC-UV method to quantify the amount of regorafenib in rat plasma using revaprazan as an internal standard. And another reseracher[11] develeloped validated RP-HPLC method for estimation of RGF MH in bulk and tablet dosage form. However, among many methods of analytical estimation, UV Spectrophotometric method persists very popular, because of it's simplicity, specificity and low cost. Nevertheless, not many or merely no method reported for RGF MH estimation by UV method. The use of UV spectrophotometer applied in the analysis of pharmaceutical dosage form has increased rapidly over the last few years because of many advantages such as excellent precision, highly sensitive, simple, fast and accurate.[12] Hence, the current investigation was designed to develop an accurate and simple UV spectrophotometric method for the estimation of RGF MH in both bulk and commercially available marketed formulations (BAY 73-4506; Stivarga; Bayer Health Care Pharmaceuticals, Inc.). This method can be effectively applied in pharmaceutical dosage form analysis, serving as a valuable tool for drug quality control and suitable for high-throughput analysis in quality control laboratories.

MATERIAL AND METHODS

Chemicals and Reagents

The pure sample of Regorafenib monohydrate (RGF MH) was obtained from well reputed research laboratory. All reagents, solvents used were of analytical grade (SD Fine Chemicals, Bengaluru, India). Double distilled water was used throughout the experiment. Commercially available tablets of RGF MH (Stivarga) with a stated content of 40 mg were bought from a local pharmacy for the study.

Instrumentation

UV-1900 UV-VIS Spectrophotometer-Shimadzu Corp/Japan was used to determine the wavelength of maximum absorbance for the study. All weighing was done on single pan electronic balance (Shimadzu).

Scanning and selection of absorption maxima

A standard stock solution of RGF MH (1 mg/ml) was prepared using methanolic HCl (Solvent A) and a mixture of acetonitrile:methanol (1:1) (Solvent B). Dilutions were then made from each standard stock solution to achieve a concentration of 10 µg/ml of the drug.

These solutions were scanned in the wavelength range of 200-400 nm against a blank using a UV-Visible Spectrophotometer to determine the absorption maxima (λmax). Clear peak absorbances were observed at 261 nm for Solvent A and 263 nm for Solvent B, as illustrated in Figure 1. Therefore, 261 nm and 263 nm were identified as the absorption maxima for Solvent A and Solvent B, respectively. Subsequently, calibration curves for estimating RGF MH were constructed at both absorption maxima (261 nm and 263 nm).

Construction of calibration curve

Standard solution of RGF MH was diluted with phosphate buffer to get dilutions containing 1, 3, 5, 7, 9, 12 and 14 µg/ml of drug. The absorbency of these solutions were measured at 261 nm against reagent blank, and the average values are presented in Table 1. A calibration curve, depicting absorbance against the concentration of the drug for RGF MH estimation, was plotted and is illustrated in Figure 2. The correlation coefficient (r) for the curve was computed. To assess linearity, a one-way ANOVA test was conducted using five randomly selected sets of calibration curves. Average absorbance values, standard deviation, and % coefficient of variance (CV) were calculated for each concentration.

Method validation

The proposed method was validated as required under ICH guidelines Q2(R1)[13,14] for different parameter such as linearity, limit of detection (LOD) and limit of quantitation (LOQ), precision, robustness, ruggedness, accuracy (recovery studies) and solution stability.

Linearity

To establish linearity and define the range, a series of drug concentrations from 1 to 14 µg/ml were meticulously prepared using standard working solvents. These solutions, prepared in triplicate, underwent absorbance measurements at 261 nm and 263 nm, with the respective solvent A and solvent B serving as blanks. The Beer-Lambert's law was adhered within the concentration range of 1 to 14 µg/ml for both methods. The concentration-versus-absorbance curve was constructed, and regression equations along with relevant statistical data were computed.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

From the data obtained in the linearity studies, the calculation of LOD and LOQ was interpreted by assessing the slope of the linearity plot. The y-intercept for each of the six replicate determinations was computed, and the standard deviation of the y-intercept was

derived. Using these values, the parameters of LOD and LOQ were determined based on the response and slope of the regression equation.

Precision

The precision of the proposed analytical method was evaluated using two measures: repeatability (intra-day) and intermediate precision (inter-day). Repeatability was assessed by assaying different concentrations of RGF MH working standard solution in three solvents. Inter-day assay precision was determined by analyzing known concentrations of the formulation three times over three consecutive days. Whereas, intra-day assay precision was done by analyzing formulation (known concentrations) in same day for three times at different time points. The precision results are expressed as % relative standard deviation (RSD), which is a measure of the variability of the assay results. The RSD values for each concentration level in each solvent are presented in Table 4.

Robustness

Robustness studies were performed to check the influence of method parameters varied intentionally on the proposed methods. The RGF MH working standard solution was diluted separately with solvents to obtain 10 µg/ml and 12 µg/mL (n=3). The absorbance of theses concentrations were measured at actual wavelength i.e., 263 nm of small varied wavelength i.e., ± 5 nm, and obtained results are interpreted in terms of % RSD.

Ruggedness

Ruggedness study is the measure of reproducibility of test results under the variations in normally expected laboratory conditions. The RGF MH working standard solution was diluted separately with solvents to obtain 10 µg/ml and 12 µg/mL (n = 3). The absorbance of theses concentrations were measured the at actual wavelength i.e., 261 nm by two different analyst and results are interpreted in terms of % RSD.

Accuracy (Recovery studies)

One commonly used method for assessing accuracy in the development of analytical techniques is through recovery studies. These studies involve adding known quantities of drugs and determining the ratio of the observed results to the expected results, expressed as a percentage. In this study, recovery experiments were conducted at three levels: 40%, 80%, and 120% of the labeled amount of the tablet. The percentage recovery was calculated in

terms of the relative standard deviation (RSD), which should ideally be less than 2%. The results of these calculations are presented in Table 3.

Solution stability

To assess the stability of the standard stock solutions of RGF MH in both solvents, experiments were conducted at various temperatures: room temperature (25°C), refrigerated temperature (2-8°C), and hot air oven conditions (45°C), for a duration of 48 h. The samples were stored in securely sealed glass containers, shielded from light. Subsequently, the standard stock solutions were appropriately diluted, and absorbance measurements were taken at both the 0 h and 48 h time intervals.

RESULTS AND DISCUSSION

Analytical method validation is the process of demonstrating that an analytical procedure is suitable for its intended purpose.[15] Literature review revealed that there is no method developed for the analysis in solid dosage form since the drug is very new. The UV Spectrophotometric method developed and validated proved to be highly specific, linear, robust and reproducible. Between pH 13 and pH 1, RGF MH is practically insoluble in water, slightly soluble in acetonitrile, methanol, ethanol, and ethyl acetate and sparingly soluble in acetone. Therefore, the stock solution was prepared by dissolving drug in proposed mediums viz., methanolic HCl (Solvent A) and acetonitrile:methanol (Solvent B) at 1:1 ratios. The maximum absorption wavelengths for solvent A and solvent B were found to be 261 nm and 263 nm, respectively, with characteristic peaks illustrated in Figure 1. Subsequently, linearity was evaluated by analyzing different concentration of standard solution of RAM MH by measuring absorbance at 261 nm and 263 nm. The Beer Lambert's law was obeyed in the concentration range of 1-14 µg/mL with regression coefficient of 0.9998 and 0.9999 for solvent A and solvent B respectively. This, clearly indicated the linearity between the concentration of analyte and absorbance values which is demonstrated in the calibration data (Table 1) and calibration curve as shown in Figure 2 for solvent A and solvenrt B.

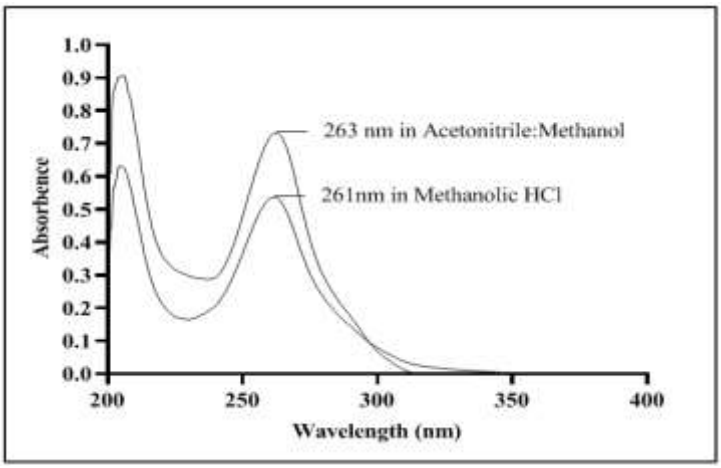

Figure 1: Absorption maxima of RGF MH in Solvent A and Solvent B.

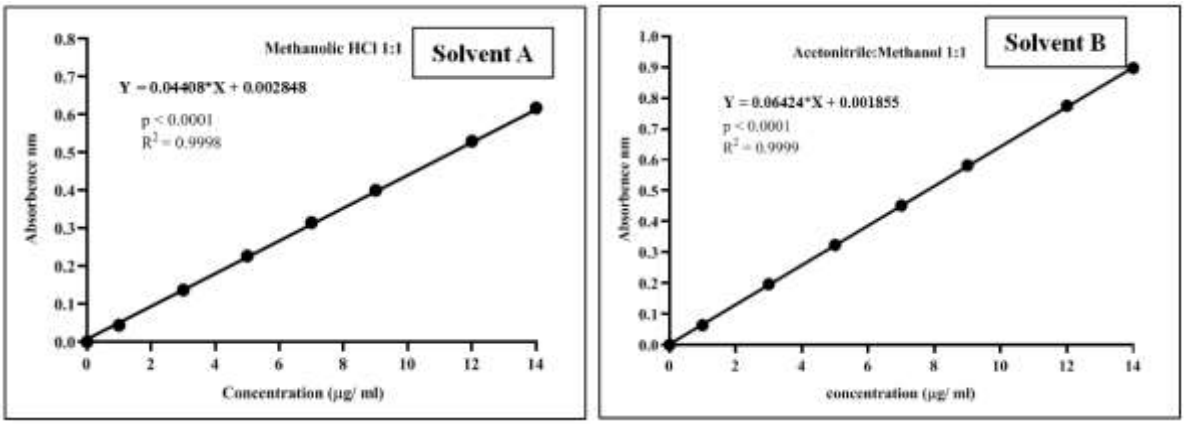

Figure 2: Calibration curve of RGF MH in Solvent A and Solvent B.

Table 1: Calibration curve data of RGF MH.

Concentration (µg/ml)	Absorbance mean ± SD (n=6)	
	Solvent A	Solvent B
1	0.044 ± 0.00057	0.064 ± 0.00057
3	0.137 ± 0.00057	0.197 ± 0.00057
5	0.227 ± 0.00057	0.324 ± 0.00100
7	0.315 ± 0.00057	0.452 ± 0.00100
9	0.400 ± 0.00115	0.582 ± 0.00057
12	0.530 ± 0.00057	0.775 ± 0.00057
14	0.618 ± 0.00057	0.897 ± 0.00057

Table 2: Statistical data of linearity curve in Solvent A and Solvent B.

Parameters	Solvent A	Solvent B
Absorption maxima (λ max)	261 nm	263 nm
Beer's law limits (µg/ml)	1 - 14	1 - 14
Molar absorptivity(ε)	4.4×10^2 L/(cm^{-cm})	6.4×10^2 L/(cm^{-cm})
95% Confidence Intervals		
Slope	0.04390 to 0.04473	0.06391 to 0.06453

Y-intercept	-0.002388 to 0.005927	-0.001163 to 0.005080
X-intercept	-0.1348 to 0.05350	-0.07941 to 0.01804
Goodness of Fit		
R square	0.9998	0.9999
P value	< 0.0001	< 0.0001
Regression Equation	Y = 0.04408*X + 0.001770	Y = 0.06424*X + 0.00195

The goodness of fit study suggested good correlation coefficient (R square - 0.9998, and 0.9999 for proposed solvent mediums) showed the validity of Beer's law with intercept response lessthan 2% calculated by least square method indicated functional linearity between the concentration of analyte and the absorbance. The P value is < 0.0001 indicated proposed method was statistically significant. Based on the standard deviation of response and slope, the LOD values for RGF MH for the proposed solvent A and solvent B were found to be 0.024 ± 0.00154 µg/ml and 0.032 ± 0.0005 µg/ml respectively. Similarly, LOQ values were found to be 0.012 ± 0.0104 and 0.009 ± 0.0010 µg/ml for solvent A and B respectively with % RSD values less than 1.

Table 3: Repeatability data of RGF MH.

Concentration (µg/ml)	Solvent A	Solvent B
	Amount recovered (µg/ml)	
10	10.1	10.0
10	10.1	10.0
10	10.0	10.1
10	10.0	10.0
10	9.98	10.0
10	10.0	10.0
Mean amount Recovered	10.01	10.01
% Recovery Mean ± SD	100.0 ± 0.5033	101.1 ± 0.1443
% RSD	0.5033	0.1427

Table 4: Intraday and Interday precision data of RGF MH.

Absorbance (nm)	Solvent A		Solvent B	
	% Recovery Mean ± SD (n=3)	% RSD	% Recovery Mean ± SD (n=3)	% RSD
Intraday precision (n = 6)				
0.284	100.3 ± 0.1400	0.624	100.3 ± 0.4157	0.145
0.549	100.7 ± 0.1607	0776	104.8 ± 4.600	0.389
Interday precision (n = 6)				
0.284	100.2 ± 0.2972	0.624	101.9 ± 0.1966	0.192
0.549	98.49 ± 0.4689	0.776	103.9 ± 1.5370	1.479

Table 3 displays the data regarding the precision of repeatability. Analytical procedure precision signifies the proximity of agreement, or the degree of scatter, among a series of measurements derived from multiple samplings of the same homogeneous sample under specified conditions. The justification for the precision of the suggested solvents is based on the recovery of RGF MH in a fixed amount through repeatability, intraday, and interday studies, as outlined in Table 4. The percentage values of RSD were all below 1, indicating that the proposed solvents are both precise and reproducible. The results of the stability study for RGF MH in the proposed methods fell within acceptable limits, affirming that solutions in the proposed methods remained stable over a 24 h period.

Table 5: RGF MH drug content data in marketed tablet formulations.

Brand name	Labelled claim (in µg/mL)	Amount recovered µg/mL)	% Recovery Mean ± SD (n=3)	% RSD
Solvent A				
STIVARGA™	6	5.87	97.70 ± 0.4840	0.4953
	8	7.83	97.42 ± 0.4319	0.4433
	12	11.95	99.44 ± 0.1652	0.1661
Solvent B				
STIVARGA™40	6	5.86	97.77 ± 0.5516	0.5641
	8	7.85	97.67 ± 0.5862	0.6001
	12	11.92	99.44 ± 0.1650	0.1659

RGF MH is marketed by the company Bayer and branded as Stivarga with a dose of 40 mg RGF per tablet. The solvents under the study were analyzed for drug content in marketed formulations viz., Stivarga™ 40 (Film coated RGF tablets) and the RGF content in marketed product and data were given in Table 5. The results were in good agreement with the label claim with % RSD values lessthan 2. Further accuracy was performed for the solvents under the study by standard addition method and the data was shown in Table 6. The percentage recovery found to be within the permissible limits with RSD values less than 2% indicated non-interference of the excipients in the formulations. Robustness evaluates the method's ability to remain unaffected by small, deliberate variations in method parameters, such as pH, temperature, or wavelength. It assesses the method's reliability under normal laboratory conditions and provides information on its tolerance to minor changes. Table 7 and 8 depicts the robustness and ruggedness of the method respectively.

Table 6: Accuracy data for marketed tablet formulations of RGF MH.

Brand Name	Amount added (µg)	% addition	Amount recovered (µg)	% Recovery Mean ± SD (n=3)	% RSD
Solvent A					
STIVARGA^{TM40}	2.4	40	2.34	98.06 ± 0.1966	0.2004
	4.8	80	4.76	97.77 ± 0.6611	0.6761
	7.2	120	7.14	99.11± 0.4479	0.4519
Solvent B					
STIVARGA^{TM40}	2.4	40	2.35	97.76 ± 0.5546	0.5673
	4.8	80	4.76	98.69 ± 0.5625	0.5699
	7.2	120	7.13	99.40 ± 0.1739	0.1749

Table 7: Robustness data of proposed methods.

λmax	Concentration (µg/ml)	Absorbance Mean ± SD (n=3)	% RSD
Solvent A			
Actual 261 nm	6	0.284 ± 0.00100	0.3521
	10	0.549 ± 0.00100	0.1821
266 nm (+5 nm)	6	0.293 ± 0.00057	0.1945
	10	0.554 ± 0.00115	0.2075
256nm (-5 nm)	6	0.275 ± 0.00150	0.5454
	10	0.547 ± 0.00057	0.1042
Solvent B			
Actual 263 nm	10	0.624 ± 0.00100	0.1602
	12	0.776 ± 0.00100	0.1288
268 nm (+5 nm)	10	0.630 ± 0.00115	0.1825
	12	0.782 ± 0.00152	0.1943
258nm (-5 nm)	10	0.618 ± 0.00057	0.0922
	12	0.771 ± 0.00057	0.0739

Table 8: Ruggedness data for proposed methods.

Parameter	Concentration (µg/ml)	Absorbance Mean ± SD (n=3)	% RSD
Solvent A			
Analyst-1	6	0.293 ± 0.00057	0.1945
	10	0.550 ± 0.00115	0.2090
Analyst-2	6	0.295 ± 0.00150	0.5084
	10	0.556 ± 0.00100	0.1798
Solvent B			
Analyst-1	10	0.630 ± 0.00115	0.1825
	12	0.783 ± 0.00152	0.1941
Analyts-2	10	0.631 ± 0.00155	0.2456
	12	0.782 ± 0.00152	0.1943

The stability assessment of the standard stock solutions of RGF MH in the specified solvents revealed consistent results under varying temperature conditions. The experiments, conducted

at room temperature (25°C), refrigerated temperature (2-8°C), and hot air oven conditions (45°C) over a 48 h period, demonstrated the robust stability of the samples. Stored in securely sealed glass containers and shielded from light, the standard stock solutions exhibited reliability in terms of stability. Diluted appropriately, the absorbance measurements taken at both the 0 h and 48 h time intervals further underscored the enduring stability of the RGF MH solutions across the tested temperature ranges.

SUMMARY

The establishment of a UV Spectroscopic method for quantifying the anticancer drug Regorafenib monohydrate (RGF MH) in bulk and commercially available formulations promises precise and reliable outcomes, presenting it as a viable alternative for routine quality control analysis. Thus, a simple, rapid and reproducible UV Spectrophotometric method for estimating of the model drug was successfully developed utilizing readily accessible solvents such as methanolic HCl, acetonitrile, and methanol. UV spectroscopic method, being a non-destructive analytical technique, proves valuable in laboratories, especially when dealing with limited or expensive samples like anticancer drugs. The absorption spectrum of RGF MH in the UV region allows for specific identification and determination, ensuring accurate estimation of the drug without interference from other formulation components. Hence, the proposed method from the present study emerges as a cost-effective choice for the routine analysis of RGF MH in both pure and commercially available dosage forms.

CONCLUSION

A novel, simple and reproducible UV spectrophotometric method for RGF MH determination have been developed in compliance with ICH guidelines. These methods, characterized by accuracy, simplicity, high specificity, reproducibility, rapid and precision, are affirmed by the successful recovery of the drug and low RSD values. The analytical reagents employed in the study are cost-effective, possess extended shelf life, and are readily accessible in any analytical laboratory. Consequently, these proposed methods offer a viable solution for effectively monitoring the drug content uniformity in tablets. Also can be used in conducting routine quantitative analysis in quality control laboratories, applicable to both pure and commercially available dosage forms.

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AUTHORS CONTRIBUTION

The collaborative efforts of all authors have successfully led to the development of a UV spectroscopic method for estimating RGF MH in both bulk and commercially available formulations. Sakriya MS, under the mentorship of Anand Kumar Y, conducted the development and validation of the UV spectroscopic technique for RGF MH. Lokamatha Swamy KM provided guidance in crafting the manuscript and structuring the research paper in accordance with the journal's guidelines.

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