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Development and validation of stability-indicating RP-HPLC method for rivaroxaban in tablet dosage form

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ABSTRACT: The goal of this study was to create an RP-HPLC method for analysing the anticoagulant rivaroxaban (RIV) and estimating associated degradants in drug and tablet dosage forms that are accurate, sensitive and validated. The analysis was carried out on a Phenomenex Luna C18 column (4.6 x 250mm, 5µm particle size) at 40°C in isocratic mode with mobile phase water and acetonitrile (45:55 v/v) at a flow rate of 1.2 ml/min and a wavelength of 249 nm. This method was validated for linearity and range, accuracy, precision, LOD, LOQ and robustness according to ICH guidelines and the findings were satisfactory. The current study was carried out under various stress circumstances in order to determine the chemical structure of the primary degradation products generated when the drug was exposed to hydrolysis (basic, acidic, neutral), oxidation and photolytic conditions. Retention time (t_R) was found to be 4.191±0.01 min. The three primary degradation products D1 (t_R shown at 1.813 min), D2 (t_R shown at 2.229 min), and D3 (t_R shown at 1.850 min) developed during stress conditions in 0.1N NaOH, 0.1N HCl and 30% H_2O_2, respectively, as revealed by chromatogram and mass spectra. Under the ion spray voltage of 5500 V for positive mode, the molecular ion [M+1] m/z 436.3 of RIV was seen. The fragment ion peak was observed at m/z 415.5, 338.6, and 149.2. In basic, acidic and oxidative environments, RIV was found to be degraded. Quadrapole MS/MS was used to identify these contaminants in drugs and drug products.

KEYWORDS: Rivaroxaban; RP-HPLC; tablets; validation; stability-indicating method.

1. INTRODUCTION

Rivaroxaban (RIV) is the first and only oral direct factor Xa inhibitor approved drug for the treatment of thromboembolic diseases [1]. RIV is a blood thinner with a molecular weight of 435.8 g/mol ($C_{19}H_{18}ClN_3O_5S$) (oral anticoagulant). It has a tiny ring of oxazolidinone that binds to factor Xa directly and reversibly [2]. In humans, RIV has a high plasma protein binding rate (92–95%) with serum albumin being the primary binding component [3]. RIV binds to the S1 and S4 compartments of serine endopeptidase, which is responsible for factor Xa inhibition potency [4]. RIV is used in adult patients undergoing knee replacement therapy and is used to treat deep vein thrombosis and pulmonary embolism. Chemical formula of RIV (figure 1) is 5-chloro-*N*-[[(5*S*)-2-oxo-3-[4-(3-oxo-4-morpholinyl)phenyl]-5-oxazolidinyl]methyl]-2-thiophene carboxamide [5]. It is a white to slightly yellowish powder that is soluble in DMF and almost sparingly dissolves in water. Only a few methods of analysis for RIV in tablet dosage forms have been published, including UV Spectrophotometry [6,7] HPTLC [8], UPLC [9], HPLC [10,11], LC/MS and TLC [12,13]. HPLC and LC-MS have been used in a few bioanalytical techniques for RIV [14-16]. Basima et. al. established a sensitive and validated HPLC method for determining RIV and associated impurities in the parent drug and its dosage forms [17]. Burla et. al. used a stability-indicating HPLC method with a UV detector to analyse RIV in bulk and tablet dosage form, but no degradation product was reported by them of RIV [18], whereas the present method shows degradation under given conditions. Effat et. al. [19] established an HPLC based stability-indicating method for RIV using the dissolution test method. Kasad et. al. published a study on RIV base degradation and technique development using RP-HPLC in Bulk [20], but in present study basic, acidic and oxidative conditions show degradation. UPLC-Q-TOF-MS/MS was used to characterise three primary degradation products for RIV under stress settings by Nathalie et. al., but they did not mention the oxidation condition [21]. Ramisetti et. al. published their work on the LC-PDA-MS/MS

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equipment for determining stability using the stress degradation approach [22]. Palandurkar et. al. published a stability-indicating study based on the analytical quality by design approach for RIV with the use of a HPTLC method [23]. According to an existing literature many published approaches include expensive initial investment, time-consuming processes, high sample analysis costs, and heavy equipment as primary problems. The development of a validated stability-indicating RP-HPLC method for estimating RIV in bulk and tablet dosage form was the focus of the research discussed here. The approach developed is extremely beneficial for identifying and characterising degradants. With the use of MS/MS spectra, we were able to anticipate potential contaminants using the fragmentation pattern pathway. RIV was quantified after being exposed to various forced degradation conditions. This knowledge will be useful in the development of synthetic, production processes as well as in determining the RIV shelf life under various conditions. For the separation and identification of degradants generated during stress investigations, the RP-HPLC approach with mass spectrometry was shown to be a very easy, simple, fast (retention time below 5 min) and useful technique.

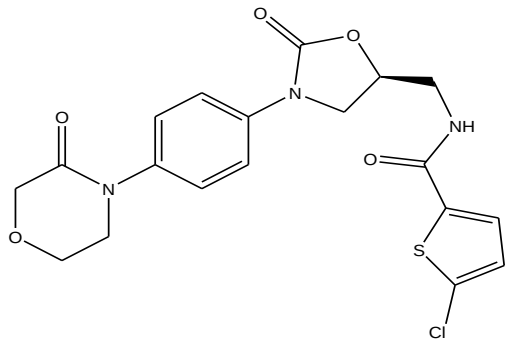

Figure 1. Structure of Rivaroxaban.

2. RESULTS AND DISCUSSION

2.1 Method development

The major goal of this study was to create a validated stability-indicating RP-HPLC method for separation of the degradants by using mass spectrometry data for elucidating the structure. The chromatographic approach was developed to investigate several relevant impurities found in RIV. RIV was injected into HPLC concurrently with all stress samples for method development. Different proportions of various solvents combinations were tested to achieve effective separation.Water and acetonitrile were chosen as solvents because of the drug's structure and nature of solubility.The best separation of RIV from its degradants was seen utilising a C_{18} column as a stationary phase and a mobile phase of water and acetonitrile (45:55 v/v) with a flow rate of 1.2 ml/min, a wavelength of 249 nm and a column temperature of 40°C with a total run time of 10 min. RIV retention time was found to be 4.191±0.01 min. Figure 2 depicted the chromatogram of RIV.

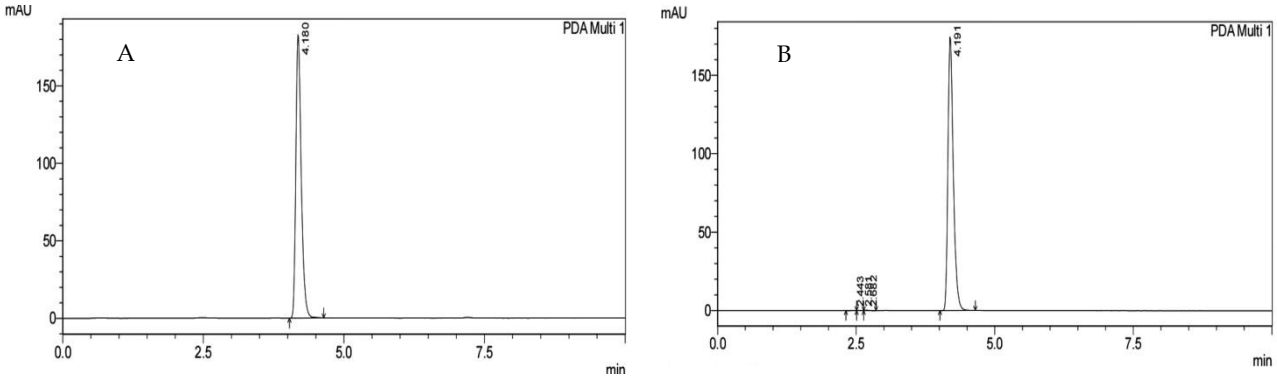

Figure 2. Chromatogram of pure RIV (A) and tablet (B)in 50 µg/mL showing t_R 4.191±0.01 min.

2.2 Method validation

According to the analysis of system suitability criteria, the HPLC equipment was suitable for the RIV chromatogram presented in table 1.

2.2.1 Linearity and range

The analytical method linearity was tested by injecting nine different level concentrations of pure RIV ranging from 1-100 µg/mL. The slope, Y-intercepts, and correlation coefficient of each RIV concentration were obtained by plotting peak area versus concentration and another with peak height versus concentration. Peak area was calculated using the linear regression equation y = 25034x – 13034, while peak height was calculated using the linear regression equation y = 3739.2x – 2212.6. In both the concentration ranges, the RIV technique was linear, with regression coefficients of 0.9999 and 0.9987, respectively. The RIV linearity curve is shown in (Figure 3; A and B).

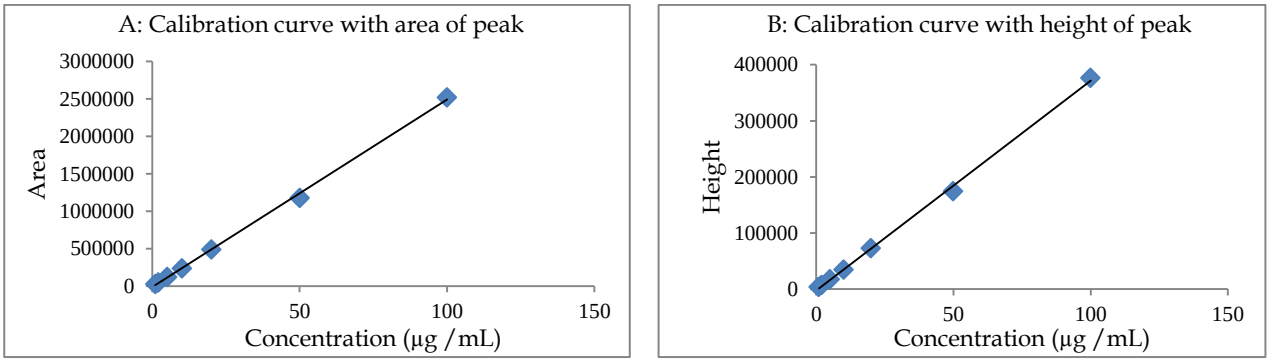

Figure 3. Calibration curve for linearity of RIV with (A) area of peak and with (B) height of peak.

2.2.2 Accuracy

The agreement between the true value and the found value determines the accuracy of an analytical method. Three concentrations were chosen from the different ranges of the RIV standard curves (12.5, 25 and 37.5 µg/mL) to determine the accuracy. The RIV recovery was tested in triplicate for 50%, 100% and 150%of the drug ingredient concentration (50 µg/mL). The impact of excipients, which are commonly found in pharmaceutical formulations with drugs, was investigated using recovery tests [24]. The recovery of RIV in the sample revealed a high level of quantitative ability. The accuracy result is presented in table 1.

Table 1. A summary of validation and regression equation parameters of the HPLC for the analysis of RIV.

Parameter	HPLC assay
System suitability	
Retention time (t_R)	4.191±0.01
Tailing factor (A_s)	1.27
No. of theoretical plate (N)	51210
Range	1-100 µg/mL
Linearity	
Regression equation	y = 25034x - 13034
Correlation coefficient	r^2 = 0.999
Accuracy at 50% level[a](mean± SD)	100.44±1.0237
Accuracy at 100% level[a](mean± SD)	99.61±0.3877
Accuracy at 150% level[a] (mean± SD)	100.89±1.0558
Precision	
Intermediate precision[b](mean±SD and RSD)	101.59±0.7471 and 0.7354
Repeatability[c](mean± SD and RSD)	101.41±1.1192 and 1.1036
Limit of detection (LOD)	0.5 µg/mL
Limit of quantification (LOQ)	1 µg/mL

[a] Average percentage recovery of three determinations.
[b] The intra-day, average of three determinations repeated within the same day.
[c] The inter-day, average of three determinations repeated over three different.
Standard deviation (SD) and Relative standard deviation (RSD)

2.2.3 Precision

The developed HPLC technique was assessed for precision study (intermediate precision and intraday research) according to ICH requirements and percent RSD was obtained from the six replicates of RIV samples. On the same day, intra-day precision was seen for RIV samples. Inter-day precision for RIV was studied for three different days. The outcomes are displayed in table 1.

2.2.4 Quantitative aspects

The signal-to-noise ratios (S/N) of 3 and 10 were used to estimate the limits of detection (LOD) and quantification (LOQ) for RIV. RIV injected had a LOD of 0.5 µg/mL and a LOQ of 1 µg/mL, shows that the drug has a better sensitivity for detection and quantification.

2.2.5 Robustness

When the chromatographic conditions like flow rate, mobile phase composition, wavelength were deliberately varied, all the analytes were adequately resolved and the order of elution was unchanged. The flow rate ±0.2 ml/min, mobile phase composition (±10%), wavelength (±2 nm) were changed. The retention time, area of peak observed and RSD was reported as per accepted range. Results for robustness are shown in table 2.

Table 2. Evaluation data for robustness study of RIV.

Robustness parameters	t_R Minute	Peak area*	% Assay ± SD	RSD
Flow rate				
Flow rate (1 ml/min)	4.182	1167128	99.6459±0.7222	0.7247
Flow rate (1.2 ml/min)	4.195	1189272	101.5365±0.5982	0.6074
Flow rate (1.4 ml/min)	4.198	1190092	101.6066±0.6228	0.6328
Mobile phase composition				
Water: Acetonitrile (40:60 v/v)	4.192	1182166	100.9298±0.4586	0.4544
Water: Acetonitrile (45:55 v/v)	4.196	1187070	101.3485±1.1261	1.1412
Water: Acetonitrile (50:50 v/v)	4.191	1183002	101.0012±1.0097	1.0198
Wavelength				
Wavelength (247 nm)	4.190	1173412	100.1825±0.3308	0.3302
Wavelength (249 nm)	4.199	1181836	100.9017±0.7735	0.7804
Wavelength (251 nm)	4.185	1180572	100.7938±0.8431	0.8498

t_R Retention time, SD Standard deviation, RSD Relative standard deviation

2.3 Degradation behavior of the drug and characterization of degradants

In alkaline, acidic and oxidative environments, RIV degrades significantly, although it remains stable in neutral and photolytic conditions. The degradation study observation table is provided in table 3. RIV mass spectra were fragmented by mass spectrometry (MS/MS) at declustering potential (DP) - 40 and entry potential (EP) - 10. Some drug product impurities or derivatives analysed as D1, D2 and D3 in different stress conditions alkaline, acidic and oxidative, according to HPLC chromatogram seen in different stress conditions and MS/MS mass spectra data. Figure 4 depicts all HPLC chromatograms and mass spectra, as well as ion fragmentation peaks. The chemical structure and fragmentation pattern of RIV within the scheme were used to interpret derivatives (figure 5). First, blank samples were subjected to the same stressful treatment as RIV samples in order to see if any excipients were found. Commercial tablet HPLC chromatograms and MS spectra were compared to blank sample HPLC chromatograms and MS spectra. The peak of RIV, MS/MS spectra in $[M+H]^+$ ESI mode was found to be m/z 436.3, which was further fragmented into m/z 149.2, m/z 415.5, and m/z 338.6. Since those are the most characteristic m/z peak found in pure RIV drug, it is proposed that m/z 149 corresponds to 3-phenyloxazolidine ion ($C_9H_{11}NO$) and m/z 338 corresponds to N-((S)-(3-(4-aminophenyl)oxazolidin-5-yl)methyl)-5-chlorothiophene-2-carboxamide ion ($C_{15}H_{16}ClN_3O_2S$)generated by breakage of the morpholinyl ring moiety.

2.3.1 Alkaline condition

RIV was refluxed with 0.1 N NaOH for 2 hours (1 mg/mL) for the alkaline condition (AK). Then, under the same chromatographic conditions, this alkaline solution (100 µg/mL) was introduced into the HPLC equipment. RIV retention time (t_R) was found to be 4.178 min. While another peak (D1) was discovered at t_R 1.813 min. The same solution was used in the ESI-MS/MS mode of the mass spectrometer

$[M+H]^+$. D1 fragmented observed as m/z 436.8, m/z 279.6, m/z 109.3 and m/z 105.3 in the mass spectrum presented in figure 4, which were not seen in pure RIV. This demonstrates the presence of degradant D1 in an alkaline medium.

Table 3. Degradation products of RIV obtained under stress conditions.

Sr. No.	Stress condition	Name of degradation Product	Retention time (min)	Exact mass	Major peak of fragmentation pattern (m/z)	Percentage of degradation
1.	0.1N NaOH	D1	1.813	436.8 (isomer)	105.3, 279.6, 109.3. 301.6	98%
2.	0.1 N HCl	D2	2.229	453.7	454, 352.6, 279.7, 106.4	80%
3.	30% H_2O_2	D3	1.850	458.6	458.6, 279.6, 106.4, 195.5	40%

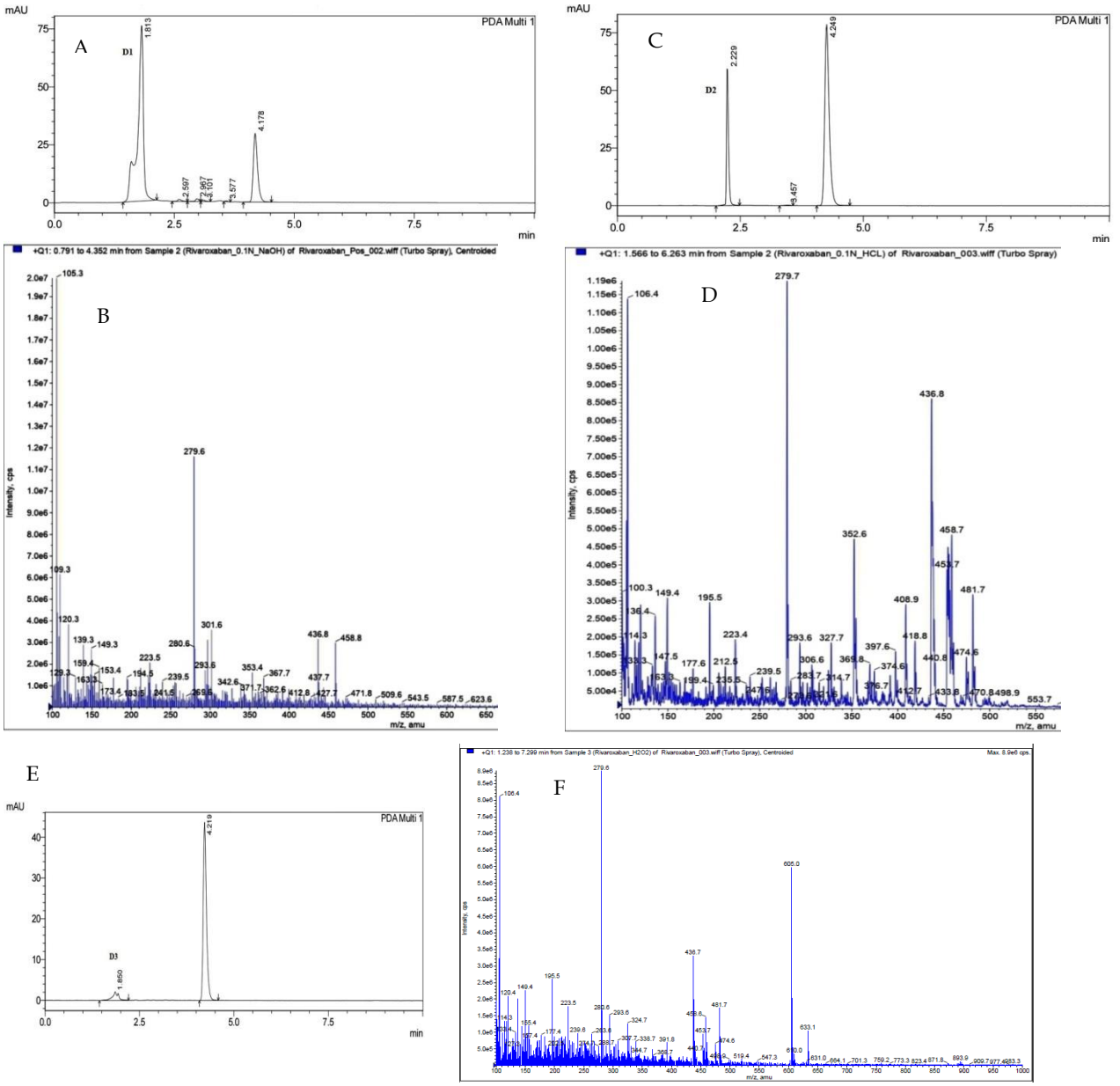

Figure 4. HPLC chromatograms and total ion MS/MS spectra scans relative to RIV stressed conditions. HPLC chromatogram of RIV exposed to 0.1 N NaOH, 80°C for 2 hr, D1 at RT 1.775 and MS/MS spectra scans having m/z 105.3, 109.3, 279.6 fragment ion observed (A and B). HPLC chromatogram of RIV exposed to 0.1 N HCl, 80°C for 4 hr, D2 at RT 2.229 min. and MS/MS spectra scans having m/z 106.4, 279.7 fragment ions observed (C and D). HPLC chromatogram of RIV exposed to 30% H_2O_2, 80°C for 8 hr, D3 at RT 1.850 min and MS/MS spectra scans having m/z 106.4, 279.7 fragment ions observed (E and F).

2.3.2 Acid condition

RIV was refluxed with 0.1 N HCl at 80°C for 4 hours (1 mg/mL) in the acidic condition (AC). Then, under the same chromatographic conditions, this acidic solution was introduced into the HPLC equipment. RIV retention time (t_R) was discovered to be 4.249 min.While another peak (D2) was discovered at t_R 2.229 min. The same solution was used in the ESI-MS/MS mode of the mass spectrometer $[M+H]^+$.D2 fragmented as m/z 436.8, m/z 352.6, m/z 279.7 (base peak), m/z 195.5 and m/z 106.4 in the mass spectrum (figure 4), which were not seen in pure RIV. In acidic solution, the major peak of RIV was found around m/z 436, m/z 454. In an acidic solution, a molecular weight of 18 Da with a water adduct molecule was detected. RIV is known to be highly vulnerable to acidic environments and degradant D2 has been identified.

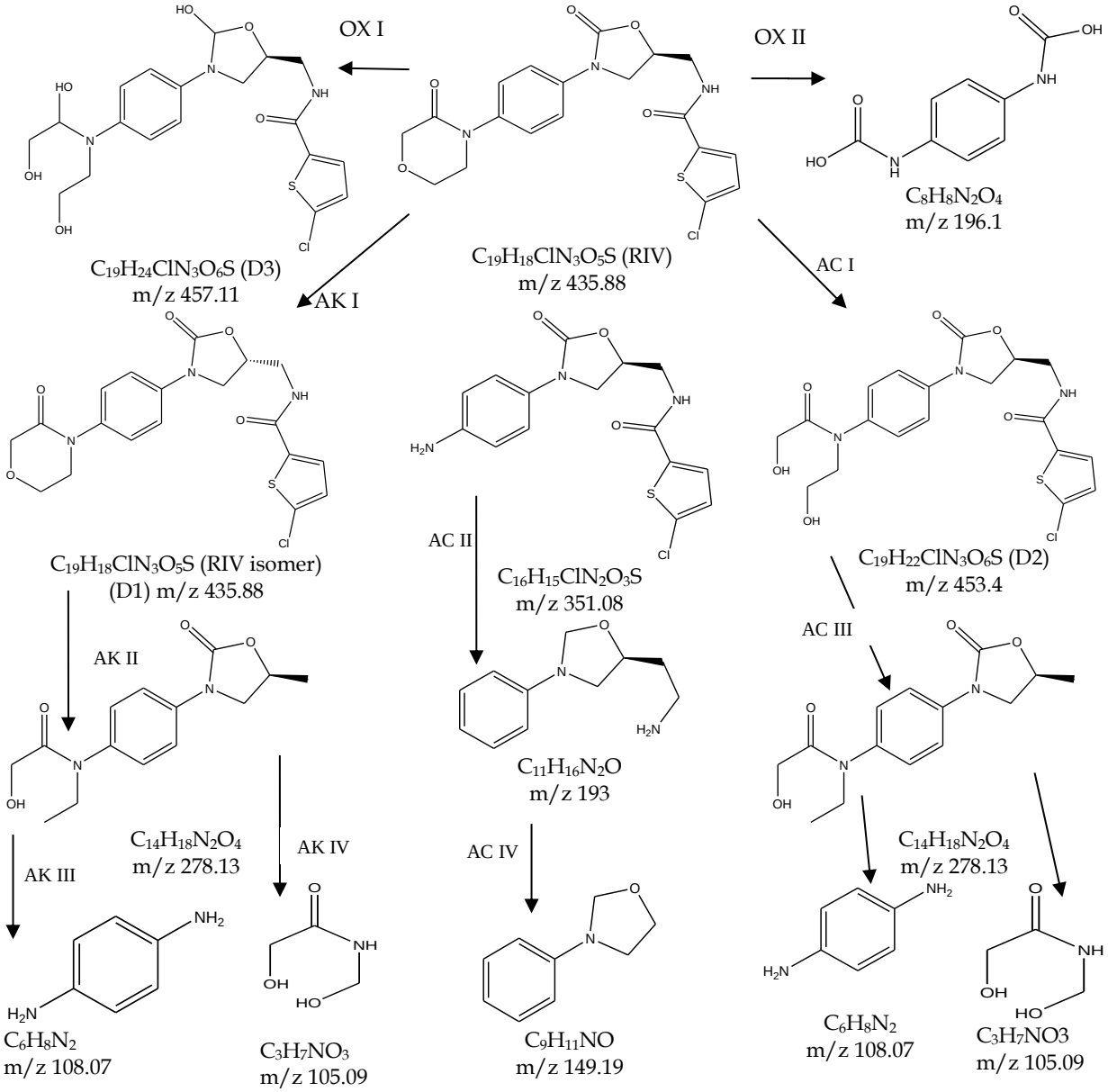

Figure 5. Structure elucidation of fragmentation pattern for the degradation products of RIV formed after drug exposal to different stress conditions such as alkaline hydrolysis (AK), acid hydrolysis (AC) and oxidation (OX) respectively.

2.3.3 Oxidation condition

RIV was refluxed with 30% hydrogen peroxide at 80°C for 8 hours (1 mg/mL) in the oxidation condition (OX). A 100µg/mL solution was prepared from this oxidised solution and injected into the HPLC instrument under the same chromatographic conditions. RIV retention time (t_R) was found to be 4.249 min.

While another peak (D3) was obtained at t_R 1.850 min. The same solution was used in the ESI-MS/MS mode of the mass spectrometer $[M+H]^+$. D3 fragmented included m/z 453.7, m/z 279.7(base peak), m/z 195.5, and m/z 106.4 in the mass spectrum presented in figure 4, which were not seen in pure RIV. Due to oxidation, this m/z 453.7 had a molecular weight of (+22 Da) was observed. This indicates that the degradant D3 was detected in an oxidation state.

2.3.4 Photochemical degradation

RIV showed no signs of degradation after being exposed to direct sunshine for 15 days. Another stressor was the usage of a UV chamber. The RIV was subjected to 200Wh/m² ultraviolet (UV) radiation and 1.2 million lux h visible light for 15 days. The RIV HPLC chromatogram did not change. Hence, it was observed that no degradation was found. This implies that the drug is stable in the presence of photochemical stress.

3. CONCLUSION

The current study investigated the method development and validation of an RP-HPLC method for the stability determination of RIV in the presence of degradation products formed under various ICH-recommended stress conditions and conceivable degradation product structures. In three different stress situations identified by high-resolution ESI-MS/MS techniques, the RP-HPLC approach proved to be an extremely powerful tool for the characterization and identification of the three major degradation products D1, D2, and D3 of the anticoagulant drug RIV. It was found to be degraded in basic, acidic and oxidative environments. The drug was found to be photolytically stable. LOD, LOQ, linearity, robustness, accuracy and precision were tested as part of the validation process for the proposed method. This approach was used to test a commercial RIV tablet formulation. The proposed approach is accurate, rapid, inexpensive, and does not require chemical processes or wet chemistry, making it a comparatively simple, rapid, and environmentally friendly method for routine analyses. The discovery of RIV degradation products opens new avenues of research and knowledge on drug stability and provides more tools for quality control and safer therapies.

4. MATERIALS AND METHODS

4.1 Chemicals and reagents

Neuland Laboratory Limited, Hyderabad, India, supplied RIV as a gift sample. Throughout the analysis, all HPLC grade solvents were employed, including water (Fisher scientific, India) and acetonitrile (Fisher scientific, India). RIV tablets "Xavian 10" (Indus Pharmaceuticals Ltd., Sikkim, India) and "Xerelto" (Bayer India Limited, Mumbai, India)were procured from a local pharmacy store. The content of RIVwas 10 mg/tablet.

4.2 Instrument

All of the analyses were carried out on a Shimadzu HPLC with an SPD- M20A prominence diode array detector (Shimadzu, Japan). The Autosampler SIL-HTC, a column oven (CTO-10 ASVP) and a Binary pump LC 20 AD was employed. The DGU-20A3 Prominence degasser was used for degassing the solutions. The LC Postrun Analysis software was used to collect and process the data. A stainless steel C_{18} column (Phenomenex Luna) was chosen; it was packed with octadecyl silane and had ligands bonded to the silica surface with a particle size of 5µm and a dimension of 4.6 X 250mm. Shimadzu LC 20AD LC system with 4000 QTrap analyzer SCiex, MDS SCIEX equipped with electrospray ionisation method was used for mass instrument. Data was collected and processed using Analyst (version 1.4) software. A photostability chamber equipped with a UV lamp(Vilber Lourmat, France, 6w, VLC), a photodegradation investigation was carried out. The light had a 200Wh/m² output and 1.2 million lux h intensity. Throughout the analytical study, a weighing balance (Shimadzu Corporation Japan, AX200) with a minimum of 0.1 mg and maximum of 200 g capacity was used.

4.3 Chromatographic conditions

The chromatographic stationary phase was selected as C_{18} column (4.6 x 250mm dimension and 5µm particle size) at the column temperature 40°C. The isocratic mobile phase containing solvent A water and solvent B acetonitrile (45:55 v/v) was used. The mobile phase was filtered through 0.45 µm membrane filter

and degassed by ultrasound sonicator for 10 min before use in the analysis. The flow rate of the mobile phase was 1.2 ml/min. The volume of injection was 5 µl. Wavelength scanning was performed on PDA detector and wavelength of 249 nm was selected for analysis. Mass spectrometric conditions turbo ion spray interface (TIS) operating in the positive ionization mode was used in the study. Declustering potential (DP) was 40 and entrance potential (EP) of 10 was used. The pressure of the drying gas (nitrogen) was 35 psi and the temperature was 500^0C. The ion spray voltage was set at 5500 V for positive mode.

4.4 Preparation of analytical solutions

The standard stock solution (1 mg/mL) was prepared by placing an accurately weighed quantity of RIV standard in a 10 ml volumetric flask with some diluents (acetonitrile and water 80:20 v/v), shaking well, and adding diluents with a concentration of 1000 µg/mL. The stock solution was diluted, well mixed and used to test all RIV validation parameters. Solvent A (water) and Solvent B (acetonitrile) were used to make the mobile phase (45:55 v/v). After 15 minutes of sonication, the mixture was filtered through 0.45 µm membrane filter paper. The average weight was estimated after weighing twenty RIV tablets. To make fine powder, the tablet was triturated in a dry and clean mortar. In a 10 ml volumetric flask, an amount of powder containing 10 mg of the drug was weighed and dissolved in minimal diluent, mixed well and agitated for 5 min. A sufficient amount of solvent was added to make volume up to 10 mL of 1000 µg/mL concentrated solution. For further research study, sample solutions were diluted appropriately from above solution. The 0.45 µm membrane filter paper was used to filter all of the solutions.

4.5 Validation of Proposed Method

4.5.1 Linearity and range

For the linearity test, different series of RIV solutions were prepared from standard stock solutions and diluted with diluent. Six distinct concentration levels (i.e. 1, 5, 10, 20, 50, 100 µg/mL) were selected and the concentration range of 1.00-100.00 µg/mL was fixed. Peak area and peak height were subjected to least square regression analysis to create a calibration equation with slope, Y-intercepts, and correlation coefficient in order to assess linearity (r^2).

4.5.2 Accuracy

Recovery trials make a use of a standard addition method to a laboratory-made synthetic combination to determine the method accuracy in triplicate. Known amounts of RIV samples were added to a constant weight 50 µg/mL synthetic mixture at three distinct concentration levels (50%, 100%, and 150% of label claim). Diluents were used to make up the volume of diluted samples. Peak area and peak height were used to estimate the recovery.

4.5.3 Precision

The proposed method's repeatability was tested by testing six replicates of RIV standard mixing solution. Using equipment housed within the same laboratory, the intermediate precision of the test procedure was examined on separate days and by different analysts. For precision, the % RSD of the RIV peak areas was determined.

4.5.4 Sensitivity

By introducing dilute solutions of known concentrations, the limits of detection (LOD) and limit of quantification (LOQ) for RIV were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively.

4.5.5 Robustness

The effect of minute but deliberate modifications in optimum chromatographic conditions developed for RIV was investigated using a robustness study. The different modification included a 10% change in flow rate, a ±2nm wavelength shift and a 2% change in the mobile phase composition. It was tested with three samples of RIV (50µg/mL) under all of the above conditions. Under all adjusted conditions, all system suitability characteristics and changes in conditions were compared.

4.5.6 Solution stability

Stress degradation studies are required for novel drug substance stability testing in order to determine the intrinsic stability characteristics of active drug components. This is especially critical when dealing with active drug content, which necessitates precise and accurate dosing. A validated stability-

indicating analytical procedure is required throughout the early stages of preformulation investigations for generic products. It ensures the active ingredient's stability throughout the formulation, production and storage processes. Analysis processes that are well-designed can assist to avoid the introduction of sub-standard medicines into local markets.

4.6 Application to pharmaceutical formulations

The average weight was established after twenty tablets (Xavian 10) were correctly weighed. To make a fine powder, the tablets were crushed in a mortar. A quantity of RIV equal to 10 mg was collected and dissolved in diluents (acetonitrile: water, 80:20 v/v). A Whatman filter paper was used to transfer the mixture into a 100 ml volumetric flask. To prepare 50.00 µg/mL concentrations, the sample solution was diluted appropriately. For 10 min., the diluted solution was sonicated for degassing. Before being evaluated with the proposed analysis approach and the reported procedure, it was filtered through a 0.45 µm membrane filter.

4.7 Stress degradation study

The degradation tests were performed in accordance with ICH recommendations. RIV and its tablet formulation were subjected to a variety of degradation conditions, including hydrolytic (alkaline, acidic, neutral), oxidative and photolytic degradation. Through the research of RIV degradation in RP-HPLC, the duration of exposition to degradation conditions and the molarity of acid and alkaline mediums were measured at a specific temperature. Preparing RIV solutions (1 mg/mL) in 0.1 N HCl and refluxing at 80°C for 4 hours, 0.1 N NaOH with refluxing at 80°C for 2 hours and distilled water with refluxing at 80°C for 4 hours were used for hydrolytic degradation study. RIV solution (1 mg/mL) was prepared in 30% hydrogen peroxide and refluxed at 80°C for 8 hours for oxidative experiments. A layer of solid dry powder of RIV was exposed to direct sunlight for 15 days to conduct photolytic tests. The drug was kept in a petri dish on the platform. For the degradation investigation, UV light of 200Wh/m^2 and visible light of 1.2 million lux h were used for 15 days. All of these stress conditions were used until considerable deterioration was obtained. Before the analysis, all the degradation study samples were collected and diluted to the appropriate concentration. For testing, this solution was injected into the HPLC instrument. The RIV assay of the stressed samples was compared to the standard untreated drug. It can be concluded from the RP-HPLC analysis of all stressed samples that the proposed analysis approach can detect all degraded products, proving the method is stability-indicating power.

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Conflict of interest statement: The authors declare no conflict of interest, financial or otherwise.

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