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Original Article

Validated Stability Indicating HPTLC Method Development for Rivaroxaban in Tablets

Hemlata Nimje[1,*] , Mayuri Magar[1], Pranita Kamble[1], Niharika Rongali[2]

[1]Department of Pharmaceutical Chemistry, JSPM's Charak College of Pharmacy and Research, Wagholi, Pune, Maharashtra, INDIA.
[2]Department of Microbiology, Pfizer India Private Limited, Visakhapatnam, Andhra Pradesh, INDIA.

ABSTRACT

Objectives: The present goal of this analytical study is to develop a simple, reliable, sensitive and robust method that can identify Rivaroxaban (RVB) by using high performance thin liquid chromatography. The current study included a degradation study by using different stress conditions in drug and tablets by using HPTLC. **Materials and Methods:** The analysis was performed by using the HPTLC CAMAG system compromising Linomat 5 sample applicator at wavelength 284 nm. Mobile phase selected as toluene (3 mL): ethyl acetate (5.5 mL): methanol (1.5 mL): ammonia (0.1 mL) with ten-minute saturation time. **Results:** The parameters like linearity and range, LOD and LOQ, accuracy, robustness, precision was studied for validation. RVB was subjected under the circumstance of forced degradation conditions like hydrolysis (base, acid, neutral), thermal, photolysis and oxidation. Using precoated silica plate as a stationary phase, the retention factor for RVB was 0.57±0.05 found. The reported HPTLC method shows a linear graph in the range of 200-1200 ng/band having regression coefficient is 0.9971. The linear regression equation was obtained as y=6.7609x+55.08. The prominent degradation shows at acidic, basic and oxidative conditions. In acidic condition drug was observed more degraded as compare to other conditions. **Conclusion:** HPTLC method is useful to identify and separate the degradant using mass spectra. No degradation was observed at thermal and photolytic conditions.

Keywords: Rivaroxaban, Tablets, HPTLC, Validation, Stability study.

Correspondence:
Dr. Hemlata M. Nimje
Department of Pharmaceutical Chemistry, JSPM's Charak College of Pharmacy and Research, Wagholi, Pune, Maharashtra, INDIA.
Email: hemanimje@gmail.com

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INTRODUCTION

Rivaroxaban (RVB) is an anticoagulant drug used to prevent blood clots.[1] In particular, it is used to prevent it from getting larger clots of blood in pulmonary emboli and atrial fibrillation as well as to prevent them after hip or knee surgery.[2] It was the direct factor oral Xa inhibitor that was readily available it is a pure (S)-enantiomer that is a white to yellowish powder that has no smell and is not hygroscopic.[3] It affects blood clotting at a critical stage by decreasing prothrombin's activity and perhaps both free and coagulation factor Xa which is clot-bound, therefore effective blockage of thrombin production results in an extension of the clotting time 8-12 hr. Factor Xa in the prothrombinase complex is inhibited by RVB both free and bound.[4] RVB is not soluble in water and in organic solvents, RVB just slightly soluble. It is chemically 5-chloro-*N*-[[(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl]-1,3-oxazolidin-5-yl] methyl] thiophene-2-carboxamide having 435.89 g/mol molecular weight and $C_{19}H_{18}ClN_3O_5S$ molecular formula.[5] The structure of RVB was depicted in Figure 1. Bhavyasri *et al.* established method development in human plasma by using UV instrument for RVB.[6] Sahithi *et al.* established development and validation on RVB by using a UV-spectrophotometer.[7] Nimje *et al.* established stability indicating method on RVB by RP-HPLC in tablets.[8] Sayeda *et al.* reported an analytical method for RVB by RP-HPLC assay procedure.[9] Sankar *et al.* established validated RP-HPLC method for RVB in its pure form.[10] Pathan *et al.* established development and validation by LC-MS method on RVB for human plasma.[11] Oliveira *et al.* worked on LC-MS/MS instrument and developed sensitive method in plasma for quantification of RVB.[12] Alam *et al.* established ecofriendly HPTLC method using green solvents in nanoparticle formulations for RVB estimation.[13] Shukla *et al.* established HPTLC method in human plasma.[14] Palandurkar *et al.* studied HPTLC method by quality risk assessment design of expert software for identification of RVB but in present study stability indicating method development and force degradation study on RVB reported.[15] Method development and stability study on RVB by HPTLC not reported in detail earlier. Hence, it was thought that the HPTLC method was developed for RVB and validated as per regulatory (ICH) guidelines. The stability studies with different degradation conditions were studied. The interpretation of degradant in acidic condition was identified with MS/MS spectra. The present HPTLC method for RVB can

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be used for quality control tests because it is easy to use, precise, quick and sensitive.

MATERIALS AND METHODS

Chemicals and Reagents

RVB was provided by Neuland Laboratories Limited, Hyderabad, India. All HPLC grade solvents were employed throughout the complete analysis including methanol toluene, ethyl acetate and ammonia from Loba Chemie, India. RVB Tablets "Xavian 10" and "Xarelto 10" (Indus Pharmaceuticals Ltd, Sikkim, India) were purchased from a pharmacy shop. The contents of tablets were 10 mg per tablet.

Instrumentation and Chromatographic Conditions

The instrument used for HPTLC analysis was CAMAG company with a Linomat 5 sample applicator, TLC Scanner 3 for complete analysis. The microsyringe from Linomat syringe, Hamilton was used for sample injection. The other requirements include twin trough chamber (10x10 cm) and UV chamber from CAMAG, Switzerland was used. The complete analysis was evaluated by using winCATS planar software. The stationary phase was made up of (pre-coated) silica gel 60 (E. Merck, Switzerland) with 10X10 cm dimension with 1 mm thickness. An investigation of thermal stability was done in a dry hot air oven (Bajaj OTG 2200 TM, India). Sonicators (Wensar, India), analytical balances (Shimadzu, Japan) and micropipettes (DLAB 100 L, India) are other analysis equipment.

Standard Solution Preparation

A precisely weighed quantity of 10 mg of RVB was taken to a 10 mL calibrated volumetric flask, sonicated for up to 15 min then diluted with methanol to the appropriate mark (1000 µg/mL). The prepared standard solution is filter through 0.45 µ membrane filter paper.

Sample Solution Preparation

Weighed Twenty "Xavian 10" tablets precisely to determine the average weight. The tablets were finely ground to obtain powder using a mortar and pestle for 5 min. One tablet equivalent to 10 mg of RVB was weighed accurately and shifted to a 10 mL calibrated volumetric flask with methanol as a solvent. Then, the flask contents were sonicated for around 15 min and volume up to mark was making up with methanol (1000 µg/mL). This solution was filtered through membrane filter paper of size 0.45 µ.

Optimized Chromatographic Conditions

For the chromatographic separation of the sample, aluminium plates with the dimensions 10x10 cm were employed on pre-coated with silica gel (E. Merck) having 250 µm layer. In an oven set to 60°C for 15 min, plates were dried after being prewashed with methanol. Using a Linomat 5 semi-automatic applicator, the samples were placed 15 mm from the bottom border of a TLC plate**.** The mobile phase with toluene (3 mL): ethyl acetate (5.5 mL): methanol (1.5 mL): ammonia (0.1 mL) was transferd to a twin-trough chamber and saturated for 10 min. The bands were scanned using the TLC scanner 3 in reflectance mode at 284 nm wavelength using software (winCATS). By measuring the intensity of reflected light, the peak area of the sample band was compared with that of the standard RVB band and concentrations of the sample were determined.

Validation of HPTLC Method

Linearity and Range

Using the standard stock solution, different series of RVB solutions were made for the linearity test. Different concentrations of 200 to 1200 ng/band were kept on TLC plate as a spot. After that, TLC plate developed by activation, dried and examined with use of the same chromatographic conditions. The curve was plotted by using RVB concentrations versus peak area.

Accuracy

Recovery was performed by spiking three concentrations of standard solution with 50%, 100% and 150% levels to the tablet solution (standard addition method). 300, 600, 900 ng/band of RVB were spiked to prepare tablet solution containing 600 ng/band of RVB.

Precision

For precision study, three standard solutions were used. The intermediate precision was determined on the same and different day. The intraday precision was determined by analysing the drug at three separate time intervals on the same day and the interday precision was determined by analysing the drug on three different consecutive days. Precision was confirmed by determining the percentage RSD.

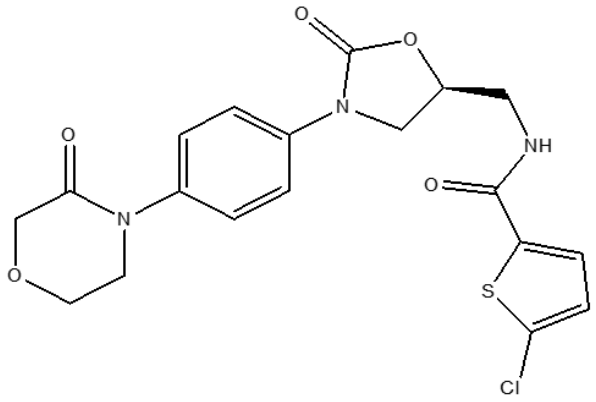

Figure 1: Chemical structure of RVB.

Robustness

Robustness was determined with the use of some small intentional changes in chromatographic conditions. The various modification using saturation time (5 min, 10 min, and 15 min), mobile phase volume (9.1 mL, 10.1 mL, and 11.1 mL), mobile phase composition changes were performed. It was tested with a standard sample of RVB (600 ng/band) for all the above conditions.

Force degradation study

According to regulatory guidelines, stress analyses were carried out to evaluate the stability- indicating assay of the proposed HPTLC technique. Intentional degradation was tried by exposing the standard solution of RVB (1 mg/mL) to the different stress conditions like 0.1 N HCl refluxed at 80°C for 2 hr (acid), 0.1 N NaOH refluxed at 80°C for 2 hr (base), 3% H_2O_2 at 80°C for 2 hr (oxidation), 60°C for 30 min (dry heat), and UV light (254 nm), distilled water at room temperature (neutral). The proposed method has the ability to measure the analyte response along with its degradation products. Before the analysis, all samples from the degradation sample were gathered and diluted to the proper concentrations. An activated TLC plate was spotted with a degradation sample, dried and then examined. The stressed sample was compared to the untreated standard drug for assay using area of peak.

RESULTS

The primary goal of present work was to develop validated HPTLC method for RVB degradant separation using MS/MS spectra to understand the structure. The chromatographic method was created to identify a number of important RVB degradants. Sample application of RVB spotted into the stationary phase made up of silica gel 60 with glass plate of size 10X10 cm dimension and 1 mm thickness. Different solvents with various volumes are used to test whether separation is efficient as per system suitability parameters. Mobile phase was used as toluene (3 mL): ethyl acetate (5.5 mL): methanol (1.5 mL): ammonia (0.1 mL) with ten min saturation time at 284 nm wavelength. Mode of separation is descending with ambient temperature, injection volume of 100 µL and band distance of 6 mm. Retention factor (R_f) of RVB was found to be 0.57±0.05 was shown in Figure 2.

Method Validation

The standard RVB solution was injected by semiautomatic sample applicator under nitrogen streamed. It was applied as a band on a single plate with a linear curve of 200 to 1200 ng/spot concentrations. The curve obtained by plotting peak area versus concentration of RVB as shown in Figure 3. A study on accuracy was carried out by spiking the sample with a known standard solution. Accuracy was accomplished by spiking three concentrations of the standard solution (50%, 100% and 150% of label claim) to the sample solution (standard addition method). The accuracy result of RVB is given in Table 1. Three sample concentration solutions were examined in order to study the precision. The intermediate precision was determined on same and different day. Precision were expressed in terms of percentage RSD depicted in Table 1. Limit of Detection (LOD) was found to be 0.169 ng/band and Limit of Quantitation (LOQ) was observed as 0.514 ng/band for RVB. When chromatographic conditions like saturation time (5 min, 10 min, 15 min), total mobile phase volume changes as 9.1 mL, 10.1 mL, 11.1 mL, mobile phase composition were deliberately varied, results were checked and peak area was observed under same chromatographic conditions, it was found that peak area unchanged. The results were reported using R_f, peak area were depicted in Table 2.

Force Degradation Study

The degradation test was performed as per ICH conditions. Various conditions like acidic, basic, neutral, oxidative, photolytic,

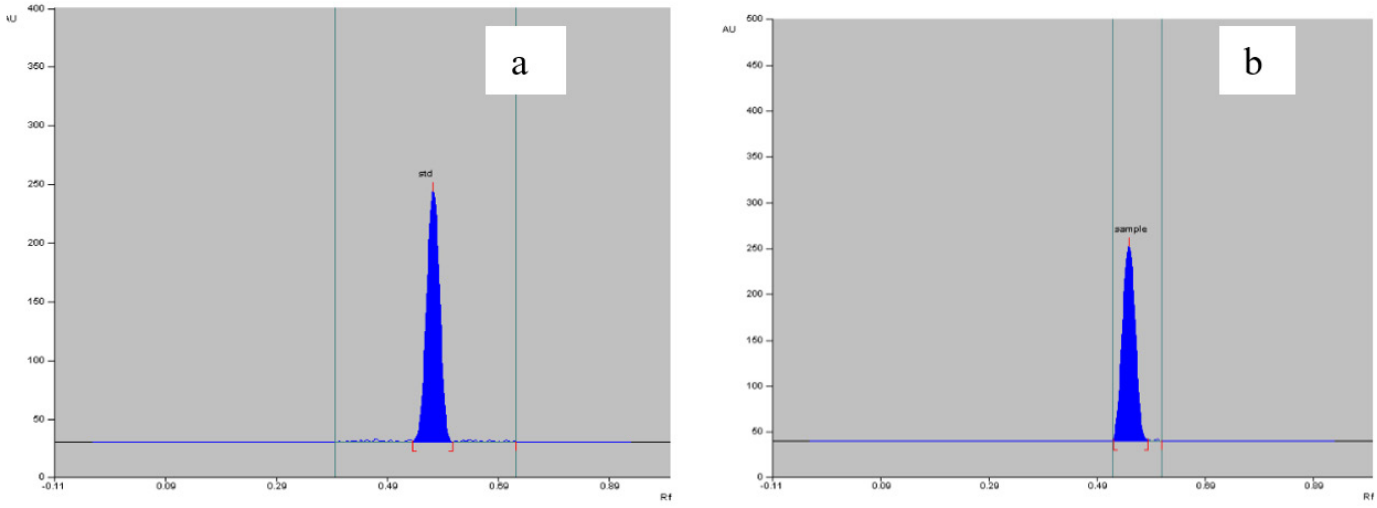

Figure 2: HPTLC densitogram of RVB (a) and tablet (b) showing R_f 0.57±0.05.

Table 1: Summary of validation for analysis of RVB.

Parameters	HPTLC Assay
Linearity range (ng/band)	200 to 1200
Linear regression equation	y=6.7609x+55.08
Correlation coefficient (r^2)	0.9971
LOD (ng/band)	0.1690
LOQ (ng/band)	0.5140
Precision (Intra-day, mean±SD and RSD)*	99.87±0.3489 and 0.3480
Precision (Inter-day, mean±SD and RSD)*	97.22±0.3533 and 0.3571
Accuracy (50% level, mean±SD)*	99.47±0.0494
Accuracy (100% level, mean±SD)*	99.54±0.3703
Accuracy (150%level, mean±SD)*	98.49±0.7815

*Replicates of three determinations, SD: Standard Deviation, RSD: Relative Standard Deviation.

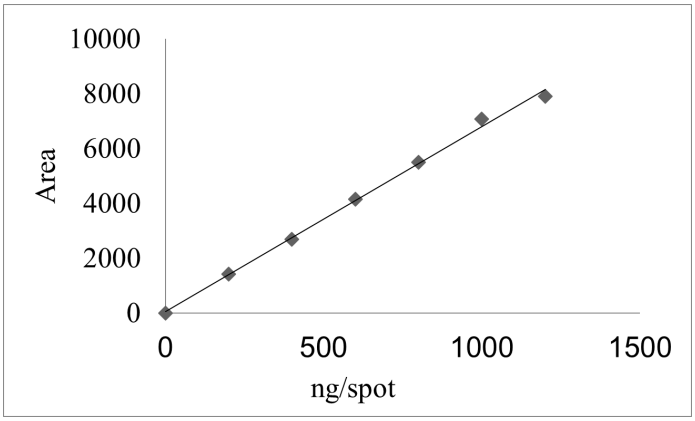

Figure 3: Calibration curve for RVB with area of peak (200 to 1200 ng/band).

thermal were applied to RVB and its tablet formulation. Prepared RVB solution of (1 mg/mL) was checked with 0.1 N HCl refluxing for 2 hr at 80°C, 0.1 N NaOH refluxing for 2 hr at 80°C, 3% H_2O_2 refluxing for 2 hr at 80°C, neutral solution at room temperature after 3 hr, thermal degradation at 60°C for 30 min, photolytic exposed for 24 hr as shown in Table 3. Using the developed chromatographic conditions, a further degradant was separated and identified by mass spectra as depicted in Figure 4. The RVB assay of the stressed sample was compared to the standard sample. Mass spectra of RVB was shown in Figure 5. In this fragmentation of RVB was found to m/z 337.1, m/z 365.1. The degradant observed at m/z 337.1 (5-chloro-N-(((S)-2-oxo-3-phenyloxazolidine-5-yl)methyl)thiophene-2-carboxamide) which is not present in standard mass spectra of RVB.

DISCUSSION

In the present work was developed and validated stability indicating method for RVB routine quality control of tablets by HPTLC. The reported literature on HPTLC for RVB detail study on qualitative analysis and degradation study not evaluated. Only one article on RVB method by HPTLC given by using QbD, was reported. In present HPTLC method, RVB was estimated in tablets along with its degradation products. The mobile phase was used as toluene (3 mL): ethyl acetate (5.5 mL): methanol (1.5 mL): ammonia (0.1 mL) with ten-minute saturation time at 284 nm wavelength. Retention factor (R_f) of RVB was found to be 0.57±0.05 which give acceptable peak shape. Hence, analysis is not time consuming. With linearity in range of concentration 200 to 1200 ng/spot of RVB which gives linear curve. The percent recovery obtained by standard addition method, was found in range of 98.523 to 99.471% indicating method is accurate and appropriate for RVB in presence of pharmaceutical excipient

Table 2: Results for robustness of RVB.

Robustness parameter	Area of peak[a]	% Assay	±SD	RSD
Saturation time (5 min)	4162	99.40	0.2026	0.2038
Saturation time (10 min)	4188	100	0.0506	0.0506
Saturation time (15 min)	4230	101.3	0.3377	0.3343
Mobile phase volume (9.1 mL)	4168	99.54	0.0675	0.0676
Mobile phase volume (10.1 mL)	4187	99.65	0.4897	0.4914
Mobile phase volume (11.1 mL)	4210	100.54	0.3387	0.3359
Mobile phase composition[b]				
Mobile phase (3:5.4:1.6:0.1 v/v)	4204	100.3	0.118	0.117
Mobile phase (3:5.6:1.4:0.1 v/v)	4093	97.7	0.219	0.224
Mobile phase (3:5.5:1.5:0.1 v/v)	4171	99.6	0.911	0.915
Mobile phase (2.9:5.6:1.5:0.1 v/v)	4163	99.4	0.236	0.237

[a]Mean of three replicates [b]Mobile phase-Toluene: ethyl acetate: methanol: ammonia.

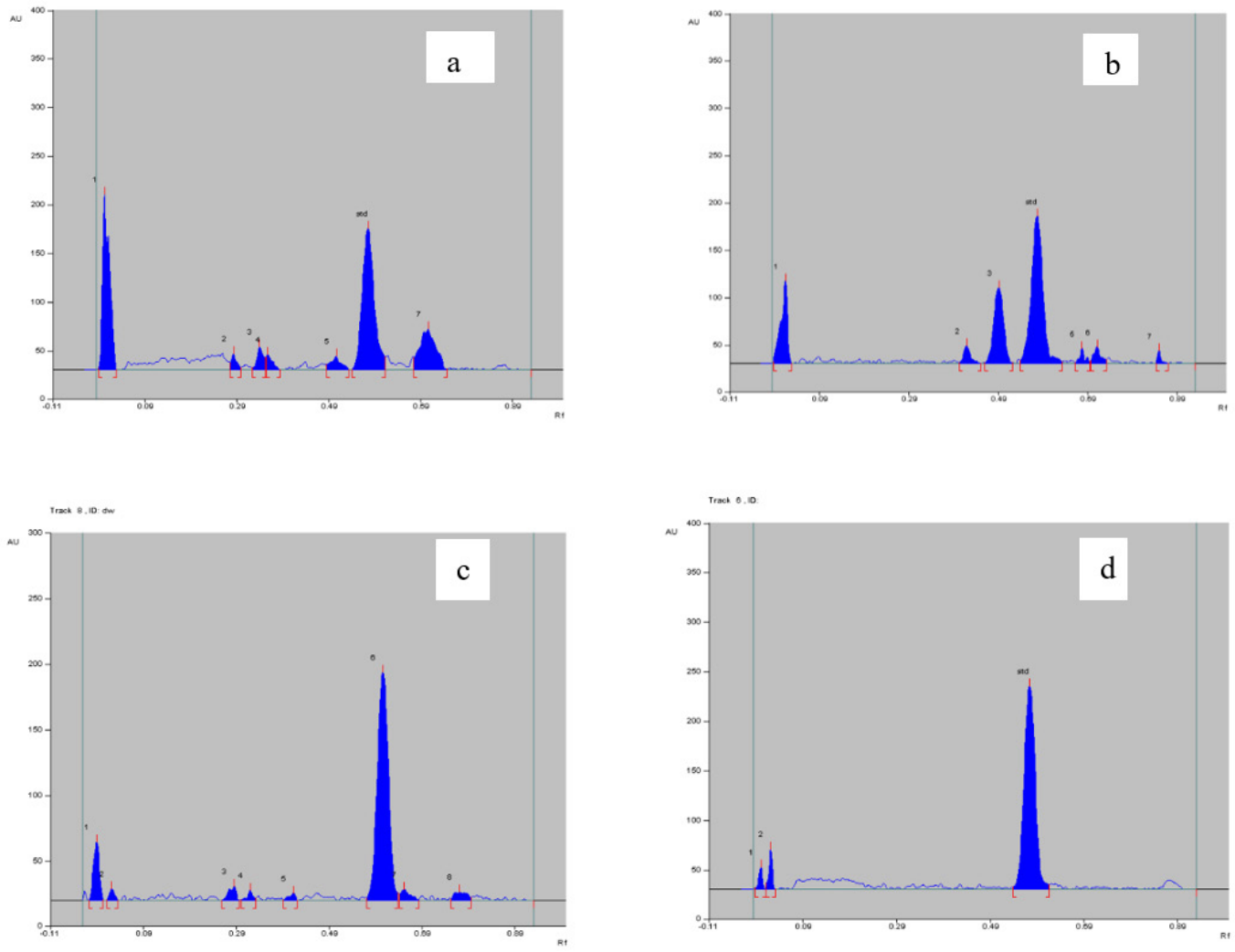

Figure 4: HPTLC chromatogram of RVB treated with 0.1 N HCl, R_f at 0.34, 0.71 (a). HPTLC chromatogram of RVB treated with 0.1 N NaOH, R_f at 0.49 (b). HPTLC chromatogram of RVB treated with water R_f at 0.2 (c). HPTLC chromatogram of RVB treated with 3% H_2O_2, R_f at 0.03 (d).

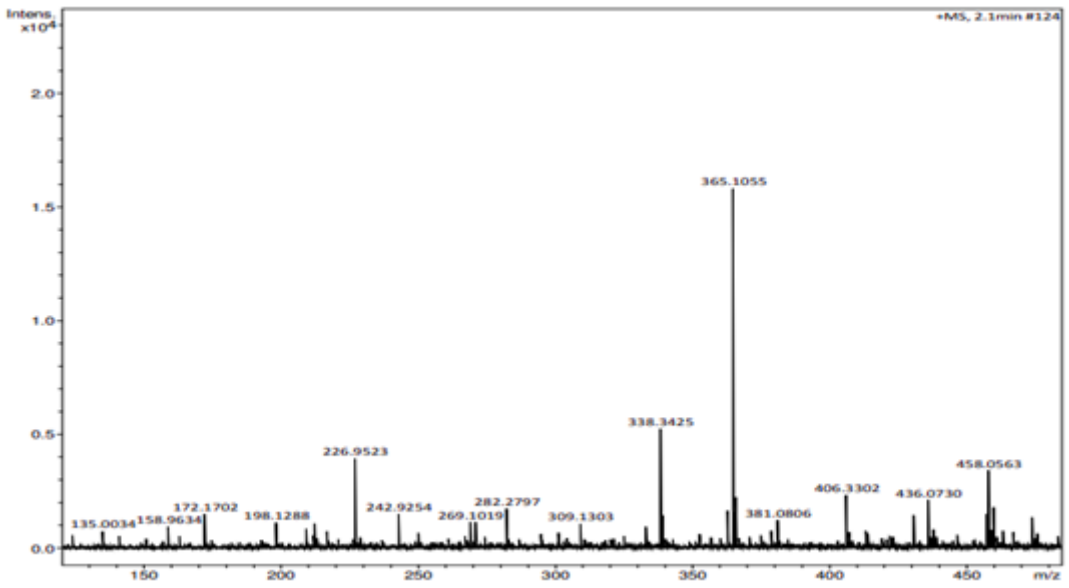

Figure 5: Mass spectra of RVB in acidic condition.

Table 3: Degradation of RVB under different stressed condition

Stress condition	Temperature (time)	R_f values of degradant	Percent degradation
0.1 N HCl	80°C (2 hr)	0.34, 0.71	25
0.1 NaOH	80°C (2 hr)	0.49	20
3 % H_2O_2	80°C (2 hr)	0.03	3
H_2O	Room Temp. (3 hr)	0.2	17
Thermal	60°C (30 min)	_	No degradation
Photolytic degradation	254 nm (24 hr)	_	No degradation

present in dosage form. The relative standard deviations for interday and intraday precision were determined to be 0.3480 and 0.3571 respectively, demonstrating repeatability and reproducibility of methods for RVB. The RVB treated under various stress condition and degradation was observed. The forced degradation was performed on RVB by using different conditions and it was found that RVB shows degradation at acid, base, oxidative condition and no degradation was observed at thermal as well as photolytic conditions. In acidic condition RVB shows maximum degradation, hence it was confirmed by using mass spectra. The fragment of RVB in acidic conditions shows at *m/z* 337.1, *m/z* 365.1.

CONCLUSION

The method development and validation by HPTLC method for the stability indicating on RVB as per various ICH guidelines was investigated in the present study. The HPTLC found to be powerful tool in separation of degradant, as more degradation was shown in acidic condition, it was identified by MS/MS technique. Drug was found to be degraded in acid, base, oxidative and neutral environment. RVB is stable in thermal and photolytic condition. Linearity, LOD, LOQ, robustness, accuracy, precision was tested for validation study. This method lowers the cost and time during the analysis of RVB because it was based on the use of a systematic analytical tool.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RVB: Rivaroxaban; **HPTLC:** High performance thin layer chromatography; **R_f:** Retention Factor; **ICH:** International conference on Harmonization; **LOD:** Limit of Detection; **LOQ:** Limit of Quantitation; **SD:** Standard Deviation; **RSD:** Relative Standard Deviation.

SUMMARY

A comprehensive literature review indicated that no HPTLC approach with stability indicating has yet to be published in detailed for the simultaneous assessment of the RVB in tablet dose form. So, the work was aimed at development and validation of a simple, accurate and precise stability indicating HPTLC method for simultaneous quantification of these compounds as bulk drugs and tablet dosage form. Various mobile phase containing ratios of toluene: ethyl acetate: methanol: ammonia was tried to separate drug. At last mobile was comprising of toluene (3 mL): ethyl acetate (5.5 mL): methanol (1.5 mL): ammonia (0.1 mL) was selected as optimal for obtaining well defined and resolved peaks for RVB. Results were discovered to be linear with a strong correlation coefficient in the concentration ranges of 200-1200 ng/band for RVB. The percent recovery obtained by standard addition method, was found in range of 98.523 to 99.471% indicating method is accurate and precise for RVB with recoveries close to 100%. When exposed to stress degradation in accordance with ICH Guidelines, drugs were discovered to be susceptible to the stress conditions used.

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