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# DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ASSAY METHOD FOR RIVAROXABAN DRUG BY HPLC

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## ABSTRACT

For small compounds, the forced degradation research is a critical analytical component of the drug development process. Stress testing is a method of demonstrating specificity in order to develop a stability-indicating analytical method using HPLC, i.e. an analytical method capable of separating degradant peaks from drug substance/drug product peaks. Stability studies are required to propose the shelf life of new drug ingredients and/or drug products according to ICH recommendations (Q1A). To estimate the shelf life of a drug substance or drug product, three types of stability tests must be completed: accelerated stability (ACC), intermediate stability (INS), and regulated room temperature (CRT) stability. The intrinsic stability of the molecule is determined by a stability analysis, and it is expected that the drug ingredient will decompose and form additional molecules known as contaminants. Various stress conditions are used in forced degradation research to degrade/decompose the main component and generate impurities, which should separate from the main compound and each other. As a result, forced degradation studies are used to estimate the degradant/decomposed impurities that occur during stability tests and to suggest the shelf life of new drug ingredients and/or drug products.

**KEYWORDS:** HPLC, Stress conditions, Stability, Analytical method, Validation.

## INTRODUCTION

Rivaroxaban is an anti-clotting medicine that works at a critical moment in the clotting process to prevent blood clots from forming.[1-2] It's also used to help people with atrial fibrillation who don't have heart valve damage prevent strokes or major blood clots. It works by inhibiting the function of a natural chemical that aids in the formation of blood clots.[3-5]

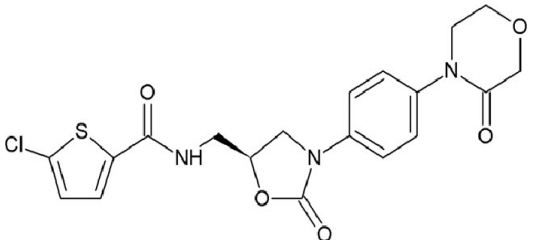


**Figure no. 01: Chemical structure of rivaroxaban.**

Rivaroxaban is a (S) Enantiomer in its purest form. It's an odourless, non-hygroscopic powder that ranges from white to yellowish in colour. Water and aqueous media are nearly insoluble in it. Rivaroxaban's molecular structure For the determination of rivaroxaban in tablets, an RP-HPLC method was devised in this work. ACN: Water (55:45 v/v) mixture was used to achieve isocratic elution.[6] UV detection was at 249 nm with a flow rate of 1.2 mL min-1. Within 2.21 and 3.37 minutes, the internal standard (Caffeine) and rivaroxaban eluted. According to ICH criteria, the devised approach was found to be linear in the range of 0.005 to 40.0.

## MATERIALS AND METHODS

The analysis was performed on a Macherey-Nagel Nucleodur C18 column (250 4.6 mm, 5 m particle size) with a mobile phase of acetonitrile and water in a gradient programme at a flow rate of 1.5 mL/min, a column oven temperature of 55°C, and a UV detector wavelength of 254 nm.[7] Proton nuclear magnetic resonance and mass spectrometry with HPLC were used to characterise the rivaroxaban standard and impurities. J T Baker provided HPLC quality acetonitrile and methanol. Merck chemicals provided analytical quality potassium dihydrogen phosphate and orthophosphoric acid. Throughout the analysis, HPLC grade water from the Millipore system was used. Ranchem's ion pair reagent Octane sulfonic acid was acquired.[8]

### Chromatographic condition

Buffer was made with 0.02M anhydrous potassium dihydrogen phosphate and 1 gramme Octane sulphonic acid solution, which had pH 3.0 adjusted with orthophosphoricacid. Acetonitrile: Methanol (820:180v/v) was used to make the solvent combination.[9-12]

### System suitability

It's an essential component of the chromatographic system. Resolution, capacity factor, tailing factor, theoretical plate count, relative retentions, and other parameters are calculated and compared to the system's standard specifications.[13-17]

**Specificity**

The capacity to assess the analyte definitively in the presence of components that might be present. The effect of excipients and other additives that are often included in the formulation is determined by the specificity of an analytical approach. In the Assay parameter, the test findings were compared to the results of a standard medication.[18-22]

**Linearity**

The capacity to produce test findings (within a certain range) is directly related to the concentration of analyte in the sample. Visual evaluation of the plot of signal as a function of analyte concentration is used to determine linearity. If there is a linear relationship, the regression line is used to generate the test results using the least squares approach. For Rivaroxaban, the method's linearity was tested at six concentration ranges ranging from 10 to 40 g/ml.[23-28]

**Accuracy**

The measure of how close the experimental value is to the true value' is the definition of analytical technique accuracy. Standard addition was used to determine the method's correctness. To the pre-analyzed injectable solution, a known amount of standard medication is added.[29]

**Precision**

The degree of scatter between a set of measurements obtained from serial sampling of the same homogeneous sample under the specified conditions. There are three levels of accuracy: repeatability, intermediate precision, and reproducibility. Rivaroxaban intra-day precision study was conducted by estimating correspondence replies on the same day with three different concentrations, while Rivaroxaban inter-day precision study was conducted by calculating correspondence responses on three distinct days with three different concentrations.[30]

**Robustness**

The robustness of an analytical procedure is a measure of its ability to remain unaffected by modest but deliberate changes in method parameters, and it indicates its reliability in routine use. The assessment of robustness should be done throughout the development phase and is dependent on the technique under investigation. It should demonstrate the accuracy of an analysis in the face of deliberate changes in technique parameters. The robustness of the

suggested approach is determined by varying the mobile phase flow rate and wavelength.[31-34]

## RESULTS AND DISCUSSION

### Method development

### Optimized chromatographic conditions

**Table no. 01: Optimized chromatographic conditions.**

| | |
|---|---|
| ➢ Mobile Phase | ➢ Methanol : water (50 : 50) |
| ➢ Column | ➢ C18, 4.6 mm x 1.5 cm , 5 µm (Make-Shimadzu) |
| ➢ Flow rate | ➢ 0.80 ml/min |
| ➢ Injection volume | ➢ 10µl |
| ➢ Wavelength | ➢ 250 nm |
| ➢ Run time | ➢ 8 min |

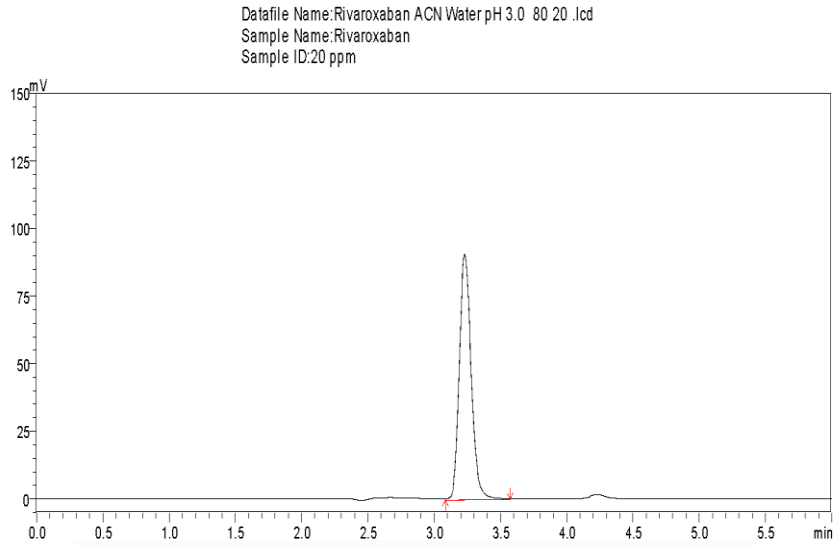


**Fig. no. 02: HPLC chromatogram of rivaroxaban.**

### Solution preparation

Weighed 100 mg of Rivaroxaban and dissolved it in 100 ml of mobile phase -Stock solution (1000 ppm) 1 ml of Stock solution diluted to 100 ml with mobile phase. (10 ppm)

**Table no. 02: Optimized chromatographic results.**

| Peak name | Retention time | Area | Area % | Asymmetry | Theoretical plates |
|---|---|---|---|---|---|
| Rivaroxaban | 3.246 | 768392 | 100.00 | 1.169 | 5553 |

**Precision**

**Inter-day- Day 1 (Conc. - 37 ppm)**

**Table no. 03: Inter-day precision Day-1 37 ppm.**

| Peak name | Retention time | Area | Area % | Asymmetry | Theoretical plates |
|---|---|---|---|---|---|
| rivaroxaban | 3.421 | 963579 | 100.00 | 1.166 | 5521 |

**Day 2 (conc. 17ppm)**

**Table no. 04: Inter-day precision Day-2 17 ppm.**

| Peak name | Retention time | area | Area % | asymmetry | Theoretical plates |
|---|---|---|---|---|---|
| rivaroxaban | 3.423 | 462992 | 100.00 | 1.71 | 5516 |

**Day 2(Conc. - 27 ppm)**

**Table no. 05: Inter-day precision Day-2 27 ppm.**

| Peak name | Retention time | area | Area % | asymmetry | Theoretical plates |
|---|---|---|---|---|---|
| rivaroxaban | 3.243 | 767464 | 100.00 | 1.167 | 5517 |

**Day 2(Conc. - 37 ppm)**

**Table no. 06: Inter-day precision Day-2 37 ppm.**

| Peak name | Retention time | area | Area % | asymmetry | Theoretical plates |
|---|---|---|---|---|---|
| rivaroxaban | 3.243 | 962732 | 100.00 | 1.167 | 5527 |

**Day 3(Conc. - 17 ppm)**

**Table no. 07: Inter-day precision Day-3 17 ppm.**

| Peak name | Retention time | area | Area % | asymmetry | Theoretical plates |
|---|---|---|---|---|---|
| RIVAROXABAN | 3.246 | 462643 | 100.00 | 1.174 | 5539 |

**Day 3(Conc. - 37 ppm)**

**Table no. 08: Inter-day precision Day-3 37 ppm.**

| Peak name | Retention time | area | Area % | asymmetry | Theretical plates |
|---|---|---|---|---|---|
| Rivaroxaban | 3.240 | 962502 | 100.00 | 1.166 | 5520 |

**Intra-day time 11:30 AM, 2.30 PM AND 5.30 PM**

**Table no. 09: Intra-day precision.**

| Level | Conc. | 11.30 AM | 2.30 PM | 5.30 PM | Mean | %RSD |
|---|---|---|---|---|---|---|
| Set 1 | 17 ppm | 463306 | 463162 | 462265 | 462911 | 0.12% |
| Set 2 | 27 ppm | 768392 | 766799 | 766277 | 767156 | 0.14% |
| Set 3 | 37 ppm | 963579 | 961978 | 961951 | 962503 | 0.10% |

**Robustness**

The robustness of an analytical technique is a measure of its capacity to remain unaffected by tiny but deliberate changes in method parameters, as well as an indication of its consistency over time. The method's robustness was determined by examining the sample at various flow rates and wavelengths.[7] Experiments with a 20g/ml concentration of Rivaroxaban were carried out under various settings, including flow rate ratio (0.1ml/min) and wavelength (+ 2nm). For deliberate adjustments in technique parameters, such as flow rate set at 0.90 and 1.10 ml/min and wavelength variation (+ 2nm) at 248 nm and 252 nm, the area of 20g/ml concentration of Rivaroxaban solution was observed.[8]

1) Change in wavelength i.e. + 2 nm.

| | Wavelength | |
|---|---|---|
| **Wl** | 248 nm | 252 nm |
| **Area** | 548394 | 555657 |

2) Change in Flow i.e. + 0.1 ml/min.

| | Flow Rate | |
|---|---|---|
| **Flow** | 0.9 ml/min | 1.10 ml/min |
| **Area** | 561825 | 547461 |

Sample preparation: 100 mg of Rivaroxaban was weighed, dissolved and diluted to 100 ml with mobile phase. (Stock solution)2 ml of above solution was transferred into 100 ml flask and diluted to volume with mobile phase. (20 ppm solution)

Change in flow rate (- 0.1 ml/min) Flow rate adjusted to 0.9 ml/min

**Table no. 10: Change in flow rate (- 0.1 ml/min).**

| Peak name | Retention time | area | Area % | asymmetry | Theoretical plates |
|---|---|---|---|---|---|
| rivaroxaban | 3.592 | 561825 | 100.00 | 1.161 | 5950 |

Change in flow rate (+ 0.1 ml/min) Flow rate adjusted to 1.10 ml/min

**Table no. 11: Change in flow rate (+0.1 ml/min).**

| Peak name | Retention time | area | Area % | asymmetry | Theoretical plates |
|---|---|---|---|---|---|
| rivaroxaban | 2,950 | 547461 | 100.00 | 1.175 | 5120 |

**Table no. 12: Change in wavelength (- 2 nm) Wavelength adjusted to 248 nm.**

| Peak name | Retention time | area | Area % | asymmetry | Theoretical plates |
|---|---|---|---|---|---|
| rivaroxaban | 3.242 | 548394 | 100.00 | 1.168 | 5523 |

**Table no. 13: Change in wavelength (+ 2 nm) Wavelength adjusted to 252 nm.**

| Peak name | Retention time | area | Area % | asymmetry | Theoretical plates |
|---|---|---|---|---|---|
| rivaroxaban | 3.241 | 555657 | 100.00 | 1.169 | 5524 |

**Accuracy**

The closeness of agreement between the value acknowledged as a conventional true value or an approved reference value and the value found is expressed by the accuracy of an analytical method. This is known as truthfulness. Accuracy was calculated using the precision experiment data, and the results for percent accuracy at three levels over the range were tallied. According to ICH guideline Q2R1, accuracy is measured across the spectrum at three concentration levels (QC standards). For each concentration level, the region of injections was determined. Percent accuracy was calculated using the measured concentrations and nominal concentrations. The same's outcomes are tabulated. Rivaroxaban results were found to be within acceptable limits of pharmacopoeial norms.

| Inj. No. | Area |
|---|---|
| 1 | 597634 |
| 2 | 595205 |
| AVG | 596419 |

Formula for assay

% Assay = Avg. area of sample solution x conc. of std x purity x 100

Area of standard solution x conc. of sample x 100

% Assay = 596419 x 20 x 100 x 100 = 101.15%

589602 x 20 x 10

Assay of Tablets – Inj. 1

| Peak name | Retention time | area | Area % | asymmetry | Theoretical plates |
|---|---|---|---|---|---|
| Rivaroxaban | 3.244 | 597634 | 100.00 | 1.170 | 5542 |

Assay of tablets- Inj 2

| Peak name | Retention time | area | Area % | asymmetry | Theoretical plates |
|---|---|---|---|---|---|
| Rivaroxaban | 3.245 | 595205 | 100.00 | 1.171 | 5539 |

| Peak name | Retention time | area | Area % | asymmetry | Theoretical plates |
|---|---|---|---|---|---|
| Rivaroxaban | 3.241 | 589602 | 100.00 | 1.169 | 5517 |

**Recovery**

Preparation of Standard Stock Solution:To make a 1000 ppm solution, 100 mg of Rivaroxaban was carefully weighed and put to a 100ml volumetric flask, where it was dissolved and diluted to volume with mobile phase.[6]

Preparation of Tablet Stock Solution: To make fine powder, ten pills were weighed and crushed. The average weight of the tablets is 0.0875 g. A 100 mL volumetric flask was filled with an accurately weighed amount of finely powdered tablets equivalent to 100 mg of Rivaroxaban; 60 mL of mobile phase was added and sonicated with intermittent shaking for 10 minutes.

**Recovery of rivaroxaban**

**Table no. 14: Recovery study of rivaroxaban.**

| Level | Spike solution area (y) | Standard solution area (m) | Conc after spike | Recovery in ppm | % recovery |
|---|---|---|---|---|---|
| 50% | 894888 | 30342 | 30 | 29.49 | 98.31 |
| 100% | 1168449 | 30342 | 40 | 38.51 | 96.27 |
| 150% | 1541427 | 30342 | 50 | 50.81 | 101.60 |

% Recovery Calculations- x = y/m

% Recovery= x * 100

T

**Stability indicating conditions**

**Forced degradation**

The forced degradation research is an important analytical component of the small molecule medication development programme. Stress testing, also known as forced deterioration, is used to demonstrate the specificity of a stability-indicating analytical method created utilising high-performance liquid chromatography (HPLC), i.e., a single analytical approach capable of distinguishing degradant peaks from drug substance/drug product peaks . Stability studies are required to propose the shelf life of new drug substances and/or drug products, according to the International Conference on Harmonization (ICH) recommendations (Q1A). Various regulatory submissions to the FDA include shelf life research. In order to estimate the shelf life of a drug substance or drug product, three types of stability studies must be conducted: accelerated stability (ACC), intermediate stability (INS), and regulated room temperature (CRT) stability. A stability research is carried out to determine the molecule's intrinsic stability, and it is expected that the drug substance/drug product would degrade/decompose and generate other molecules known as contaminants during the study. Various stress conditions are purposely used during forced degradation investigations to degrade/decompose the main component and generate impurities, which should segregate from the main compound and from each other. As a result, forced degradation studies are used to suggest the shelf life of new drug ingredients and/or drug products by estimating the degradant/decomposed impurities that would occur during stability testing. To separate and estimate all of the degradant chemicals expected to be present at the time of the forced degradation investigation, various analytical techniques/equipment can be used. HPLC-UV and HPLC-PDA are well-known techniques that are commonly utilised in the pharmaceutical industry during forced degradation experiments as part of the development and validation of the stability-indicating method. The techniques of high-performance liquid chromatography with mass spectrometry (LC-MS), gas chromatography with mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR) spectroscopy are all useful for determining the structure of degradants.

**Table no. 15: Degradation studies.**

| Degradation type | Conc of reagent | Conditions applied | Time | Remarks |
|---|---|---|---|---|
| | 0.1 n hcl | $70^0$ | 4 hrs | Observe physical appearance of sample after degradation |
| Alkali (base) | 0.1 n naoh | $70^0$ | 4hrs | |

| | | | | |
|---|---|---|---|---|
| Thermal (dry heat) | Na | 1500 | 4 hrs | |
| Uv | Expose under day light at 254nm wavelength | Ambient temperature | 24 hrs | |

**Selection and Procedures of forced degradation condition**

Forced degradation is commonly performed in several stress conditions, such as acid, alkali, peroxide, heat, and UV, together with a control sample, according to ICH guidelines and normal industry practise. There are no industry standards for how much degradation should be achieved; nevertheless, current industrial practises recommend achieving 5 to 30% degradation under any of the applied stress situations. The goal of the stress testing degradation is to replicate the control room temperature stability circumstances. The conditions or concentrations of the reagent should be optimised in circumstances where larger or lower degradations are detected. The following goals are pursued through forced deterioration studies: 1. To identify drug substance and drug product degradation pathways. 2.To distinguish degradation products associated with drug products from those produced by non-drug compounds in a formulation. 3.To figure out how degradation products are made. 4.To determine a pharmacological substance's inherent stability in formulation. 5.To determine the drug substance and drug product's degradation mechanisms, such as hydrolysis, oxidation, thermolysis, or photolysis. 6.To determine the nature of a developed method's stability.7.To comprehend medication molecule chemical characteristics. 8.To create formulas that are more stable. 9.To establish a degradation profile similar to that seen in a formal stability study conducted under ICH circumstances. The HPLC approach was used for Forced degradation tests (stress studies) of Rivaroxaban in bulk medication utilising a Schimadzu C18 (250 x 4.6 mm internal diameter; 5 m particle size) column with acetonitrile and water pH 3.0 in an 80:20 v/v ratio as the mobile phase. The component was monitored and detected with a UV detector at 250 nm at a flow rate of 1.0 ml/min.

**Acidic degradation**

Acidic decomposition Rivaroxaban was produced at a concentration of 1 mg/mL by dissolving 50 mg in 50 mL of 0.1N hydrochloric acid. In a thermostatically controlled water bath, the solution was heated to 70°C. A sample of 0.2 mL of the solution was taken and diluted with mobile phase. The sample was then examined using HPLC to determine the degree of deterioration.

**Alkaline degradation**

Alkaline decomposition 50 milligrammes of Rivaroxaban was dissolved in 50 mL of 0.1N NaOH to make 1 mg/mL Rivaroxaban. In a thermostatically controlled water bath, the solution was heated to 70°C. A sample of 0.2 mL of the solution was taken and diluted with mobile phase. The sample was then examined using HPLC to determine the degree of deterioration.

**UV degradation**

UV radiation at 254 nm was used to expose 100 mg of Rivaroxaban. After 24 hours, the sample was taken and diluted with mobile phase to obtain a 20ppm solution. The sample was then examined using HPLC to determine the degree of deterioration. Under UV light, there was no change in the appearance of the material.

**Thermal degradation**

The Petridish was held in the oven at 150°C with 100 mg of Rivaroxaban evenly scattered in a thin layer. After 4 hours, the sample was taken and diluted with mobile phase to obtain a 20ppm solution. The sample was then examined using HPLC to determine the degree of deterioration. Under thermal degradation conditions, there was no change in the appearance of the sample.

**DISCUSSION**

Rivaroxaban remains stable in higher temperatures. When compared to acid and UV degradation, it decomposed more in alkaline circumstances. The deterioration was determined to be 18.4 percent in alkaline conditions, such as 0.1N NaOH at 70°C for 4 hours. The degradation was determined to be 0.72 percent at acidic conditions (0.1 N HCl at 70°C for 4 hours). Rivaroxaban drug degradation was about 4.2 percent and 0.67 percent under UV light for 24 hours and in a heat environment at 150°C for 4 hours, respectively.

Chromatograms obtained are given below- Blank

| Peak | Name | Retention time | Area | Area % |
|---|---|---|---|---|
| 1 | Unknown | 2.612 | 2935 | 0.354 |
| 2 | Rivaroxaban | 3.237 | 824717 | 99.333 |
| 3 | Unknown | 3.819 | 2599 | 0.313 |
| Total | ---------- | | 830251 | 100.00 |

| peak | name | Retention time | area | Area % |
|---|---|---|---|---|
| 1 | rivaroxaban | 3.240 | 299655 | 100.00 |

**Alkali degradation**

| Peak | Name | Retention time | Area | Area % |
|---|---|---|---|---|
| 1 | Unknown | 2.607 | 5957 | 12.556 |
| 2 | Unknown | 3.080 | 351 | 0.740 |
| 3 | Rivaroxabn | 3.238 | 38718 | 81.606 |
| 4 | Unknown | 3.623 | 1011 | 2.130 |
| 5 | Unknown | 3.820 | 1409 | 2.969 |
| total | -------- | | 47446 | 100.00 |

Acid degradation

| Peak | Name | Retention time | Area | Area % |
|---|---|---|---|---|
| 1 | unknown | 2.495 | 4389 | 0.680 |
| 2 | Rivaroxaban | 3.237 | 618754 | 95.820 |
| 3 | Unknown | 3.818 | 22600 | 3.500 |
| Total | ------ | ----- | 645742 | 100.00 |

**UV degradation**

| Peak | Name | Retention time | Area | Area % |
|---|---|---|---|---|
| 1 | Unknown | 2.634 | 1627 | 0.245 |
| 2 | Rivaroxaban | 3.240 | 657884 | 99.280 |
| 3 | Unknown | 3.821 | 3147 | 0.475 |
| total | ------ | -------- | 662657 | 100.00 |

## CONCLUSION

The proposed study describes a new HPLC method for estimating Rivaroxaban in both bulk and tablet form. The approach was tested and found to be straightforward, sensitive, precise, and robust. The percentage of recovery indicates that the procedure is free of excipient interference in the formulation. As a result, the proposed method can be employed in normal quality control testing laboratories for routine examination of Rivaroxaban estimate.

## REFERENCE

1. Mustafa Celebier, Tuba Recber, Engin Kocak and Sacid Altinoz, RP-HPLC method development and validation for estimation of rivaroxaban in pharmaceutical dosage forms, Brazilian Journal of Pharmaceutical Sciences, 2013; 49: 360-366.
2. MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US), 2020; 24: 1. Available from: https://medlineplus.gov.
3. Badroon T and J. Sreeramulu, Development and validation of stability indicating assay by HPLC method for estimation of Rivaroxaban, International Journal of Bio-Pharma Research, 2019; 8(5): 2582-2586.

4. Basima Arous, Mohammad Amer Al-Mardini, Francois Karabet, Manal Daghestani, Fida Al-Lahham, and Alyamama Al-Askar, Development and validation of a liquid chromatography method for the analysis of rivaroxaban and determination of its production related impurities, Pharmaceutical Chemistry Journal, 2018; 52(5): 416-423.
5. Srinivasrao V, Girase YN and DiptiSoni, Development and Validation of Stability Indicating RP-HPLC Method for Rivaroxaban and Its Impurities, SOJ Biochem, 2018; 4(1): 1-6.
6. Javaid Ahmad Khan, Sheikh Abdul Khaliq, Sadaf Naeem and Muhammad Kashif, Method development and validation of solid dosage form of rivaroxaban by rp-hplc, Journal of International Research in Medical and Pharmaceutical Sciences, 2020; 15(1): 4-10.
7. Kasad Pinaz A and K.S. Muralikrishna, Method Development and Acid Degradation Study of Rivaroxaban by RP-HPLC in bulk, Asian journal of pharmaceutical analysis, 2013; 3(2): 62-65.
8. Satyendra Yadav and Nidhi Dubey, Development and validation of bioanalytical method for estimation of rivaroxaban using hplc-pda in human blood plasma, journal of drug delivery and therapeutics, 2017; 7(7): 123-125.
9. Amara Babu, Namburi L. A. Koganti, Kalyani, Palakeeti, Babji, Srinivas, Koduri S. V.Rao, Koya Prabhakara, Development and validation of UPLC method for separation and determination of rivaroxaban and its related substances in bulk drugs, Drug Invention Today, 2020; 13(4): 611-618.
10. Perzborn E, Kubitza D, Misselwitz F. Rivaroxaban, A novel, oral, direct factor Xa inhibitor in clinical development for the prevention and treatment of thromboembolic disorders, Hamostaseolog, 2000; 27(4): 282-289.
11. ROHDE, G, Determination of rivaroxaban-a novel, oral, direct Factor Xa inhibitor in human plasma by high-performance liquid chromatography tandem mass spectrometry, J. Chromatogr.B, 2008; 872: 43-50.
12. DUGGAN, S.T.SCOTT, L.J.PLOSKER, G.L, Rivaroxaban a review of its use for the prevention of venous thromboembolism after total hip or knee replacement surgery, Drugs, 2009; 69: 1829-1851.
13. TERRY,C, SUM, L. Rivaroxaban, an oral direct factor Xa inhibitor for the prevention of thromboembolism, Cardiol, 2009; 17: 192-197.
14. Kuhn J, Gripp T, Flieder T, Dittrich M, Hendig D, Busse J, UPLC-MRM Mass Spectrometry Method for Measurement of the Coagulation Inhibitors Dabigatran and

Rivaroxaban in Human Plasma and its Comparison with Functional Assays, PLoS ONE, 2015; 10(12): 78.

15. Zhang W, Lou D, Zhang DT, Zhang Y, Huang HJ, Determination of rivaroxaban, apixaban and edoxaban in rat plasma by UPLC, MS/MS method, J Thromb Thrombolysis, 1-7.
16. Celebier M, Reasber T, Koasak E, Altanajz, kars, Determination of Rivaroxaban in Human Plasma by Solid-Phase Extractionae "High Performance Liquid Chromatography, J Chrom Sci, 2015; 7: 1-5.
17. ICH guidelines, validation of analytical procedures test and methodology, Q2(R1), 2003.
18. Rockville M D. General Tests, chapter-621, chromatography system suitability United states pharmacopieial convention (USP), USP, 2016; 39.
19. Jebaliya H, Dabhi B, Patel M, Jadeja Y, Shah A, Stress study and estimation of a potent anticoagulant drug rivaroxaban by a validated HPLC method: Technology transfer to UPLC, Journal of Chemical and Pharmaceutical Research., 2015; 7(10): 749-765.
20. Vaghela D, Patel P, High performance thin layer chromatographic method with densitometry analysis for determination of Rivaroxaban from its tablet dosage form, International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6(6): 383-386.
21. Chandrasekhar K, SatyavaniP, Dhanalakshmi A, Devi C, Barik A and Devanaboyina N, A new method development and validation for analysis of rivaroxaban in formulation by RP-HPLC, Research Desk, 2012; 1(1): 24-33.
22. Pollack CV, Coagulation assessment with the new generation of oral anticoagulants, Emerg Med Journal, 2015; 0: 1–8.
23. Enriquez A, Lip GYH and Baranchuk A, Anticoagulation reversal in the era of the non-vitamin K oral anticoagulants, Europace, 2016; 18: 955–964.
24. Tissot N, Hulot JS and FunckBrentano C, Simultaneous determination of rivaroxaban and dabigatran levels in human plasma by high-performance liquid chromatography–tandem mass spectrometry, J Pharm Biomed Analysis, 2014; 100: 230–235.
25. Varga A, Șerban RC, Muntean DL, tatar CM, Farczadi L and Tilea I, Rapid liquid chromatography tandem mass spectrometry determination of rivaroxaban levels in human plasma for therapeutic drug monitoring, Revista Romana de Medicina de Laborator, 2017; 25: 145-155.
26. Rodina TA, Mel'nikov ES, Aksenov AA, Belkov SA, Sokolov AV, Prokof'ev AB and Ramenskaya GV, Development of an HPLC-MS/MS Method for Quantitative

Determination of Rivaroxaban in Human Blood Serum, Pharm Chem Journal, 2018; 52: 372– 377.

27. Harrington RA, Becker RC and Ezekowitz M, Antithrombotic therapy for coronary artery disease: The Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy, Chest, 2004; 126(3): 513–548.
28. Singer DE, Albers GW, Dalen JE, Go AS, Halperin JL and Manning WJ. Antithrombotic therapy in atrial fibrillation: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy, Chest, 2004; 126(3): 429–456.
29. Kubitza D, Becka M, Voith B, Zuehlsdorf M and Wensing G, Safety, pharmacodynamics, and pharmacokinetics of single doses of BAY 59–7939, an oral, direct factor Xa inhibitor, Clinical Pharmacology Therapeutics, 2005; 78(4): 412–421.
30. Burness CB, McKeage K. Dabigatran Etexilate, Drugs, 2012; 72(7): 963–986.
31. Bansilal S, Bloomgarden Z and Halperin JL, Efficacy and safety of rivaroxaban in patients with diabetes and nonvalvular atrial fibrillation: the rivaroxaban once-daily, oral, direct factor Xa inhibition compared with vitamin K antagonism for prevention of stroke and embolism trial in atrial fibrillation (ROCKET AF trial), Am. Heart Journal, 2015; 170(4): 675–682.
32. Mueck W, Lensing AWA, Agnelli G, Decousus H, Prandoni P and Misselwitz F. Rivaroxaban, Clinical Pharmacokinetic, 2011; 50(10): 675–686.
33. Piccini JP, Patel MR, Mahaffey KW, Fox KAA and Califf RM, Rivaroxaban, an oral direct factor Xa inhibitor,Expert Opin. Investig, Drugs, 2008; 17(6): 925–937.
34. Ezekowitz MD, Cappato R and Klein AL, Rationale and design of the explore the efficacy and safety of once-daily oral rivaroxaban for the prevention of cadiovascular events in patients with nonvalvular aTrial fibrillation scheduled for cardioversion trial: a comparison of oral rivaroxaban once daily with dose-adjusted vitamin K antagonists in patients with nonvalvular atrial fibrillation undergoing elective cardioversion, Am. Heart Journal, 2014; 167(5): 646–652.