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Original Article

# HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD WITH DENSITOMETRY ANALYSIS FOR DETERMINATION OF RIVAROXABAN FROM ITS TABLET DOSAGE FORM

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**ABSTRACT**

**Objective:** The main objective of current study was to develop and validate HPTLC, simple, precise, accurate and specific chromatographic method for the determination of Rivaroxaban from its tablet dosage form.

**Methods:** The mention drug was spotted on silica gel F254 TLC plates under pure nitrogen stream by Linomat TLC spotter. Separation was carried out by using methanol, toluene, and triethanolamine as mobile phase in ratio of 7:2.5:0.5 v/v/v. Developed TLC plates were scanned by CAMAG TLC scanner and detection was carried out at 249 nm.

**Results:** $R_f$ value of separated drug was found to be 0.60 for Rivaroxaban. The linearity of Rivaroxaban is 500 to 3000 ng/spot.

**Conclusion:** The developed method was validated as per ICH guidelines by studying various validation parameters like Accuracy, Precision, Robustness, LOD, LOQ and solvent stability. The developed and validated method was successfully applied for determination of Rivaroxaban from its tablet dosage form.

**Keywords:** Rivaroxaban, High performance Thin Layer chromatography, Densitometry analysis, Analytical method validation.

## INTRODUCTION

Rivaroxaban is chemically 5-Chloro-N-({(5S)-2-oxo-3-[4-(3-oxo-4-morpholinyl)phenyl]-1,3-oxazolidin-5- yl}methyl)-2-thiophene-carboxamide (Fig. 1)[1], it is the first available orally active direct factor Xa inhibitor[3]. Rivaroxaban is an oxazolidinone derivative optimized for inhibiting both free Factor Xa and Factor Xa bound in the prothrombinase complex[2]. Inhibition of Factor Xa interrupts the intrinsic and extrinsic pathway of the blood coagulation cascade, inhibiting both thrombin formation and development of thrombi.

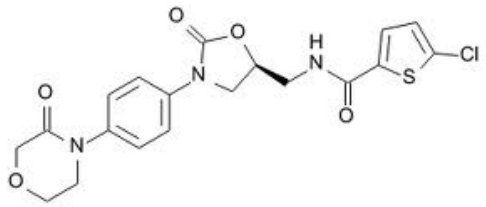


**Fig. 1: Chemical structure of Rivaroxaban**

The tablet of Rivaroxaban is available in dose of 10 mg of Rivaroxaban and Excipients q.s Colours (ferric oxides USPNF –red &titanium dioxide-white IP). To date, there are no official pharmacopoeia monograph of research paper which indicates determination of Rivaroxaban by High performance thin layer chromatography.

Review of literature revealed that various spectrophotometric methods [4-7], bioanalytical methods involving UPLC-MS/MS[8],UHPLC- MS MS[9],colorimetric method[10],RP-HPLC[11-14] have been reported for the estimation of Rivaroxaban from its tablet dosage form.

The review of literature revealed that there is no HPTLC method has been reported for the estimation of Rivaroxaban from its tablet dosage form. The present paper describes a simple, accurate and precise method for estimation of Rivaroxaban in its tablet dosage form by high performance thin layer chromatographic method (HPTLC). The developed method was validated in accordance with ICH Guidelines[15]. Finally the developed method was successfully applied for determination of tablet containing 10 mg of Rivaroxaban.

## MATERIALS AND METHODS

### Materials

Rivaroxaban reference standard was procured from Bayer Zydus Pharma Pvt.Ltd (99.9% Pure) as gift sample for research purpose. Tablets containing Rivaroxaban 10 mg was procured from local market (Xarelto). Silica Gel F254 plates was purchased from E Merck India Pvt. Ltd. Mumbai. Methanol, toluene, and triethanolamine were purchased from S.D. fine Chemicals.

### Instrument and Experimental Conditions

HPTLC analysis was carried out on silica gel 60F254 HPTLC plates (10 × 10 cm) by means of a Linomat V automatic spotter equipped with a 100 µl syringe and operated with settings of band length, 6 mm; distance between bands, 5 mm; distance from the plate edge, 10 mm; and distance from the bottom of the plate, 10 mm. The plate was developed in a twin-trough chamber previously saturated for 20 min with the mobile phase for a distance of 7 cm. For densitometry analysis, the spots on the air-dried plate were scanned with the Scanner III at nm using the deuterium source. Photograph of developed plates were taken by REPROSTAR camera at 254 nm.

**Table 1: Optimized Chromatographic conditions**

| Parameter | Condition |
|---|---|
| Mobile phase | Toluene: Methanol: Triethanolamine(v/v/v) |
| Diluent | Methanol |
| Stationary phase | Silica gel G F254 |
| Distance run | 70 mm |
| Chamber dimensions | 20 x 20 cm |
| Saturation time | 20 minutes |
| Temperature | Ambient |
| Detection wavelength | 249 nm |
| $R_f$ value | Rivaroxaban: 0.602 ± 0.0083 |

**Preparation of working solutions**

Based upon trial and error at laboratory scale finally it was decided to prepare stock solution of 500 mcg/ml of RRB.

Final mobile phase consisting of methanol, toluene, and triethanolamine mobile phase in ratio of 7:2.5:0.5 v/v/v was placed in CAMAG TLC chamber and saturation was performed for 20 min. Final Separation was achieved using above mobile phase.

**RESULT AND DISCUSSION**

**Analytical Method validation**

**Rivaroxaban (RRB) standard stock solution: (500 ng/µl)**

Standard 50 mg of RRB was weighed and transferred to a 100 ml volumetric flask and dissolved in Methanol. The flask was shaken and volume was made up to the mark with Methanol to give a solution containing 500ng/µl RRB.

**Calibration curve for the RRB (500 to 3000 ng/spot)**

Appropriate volume of aliquot from standard RRB stock solution was filled in the syringe and under nitrogen stream by a semiautomatic sample applicator; it was apply in form of band on a single plate having concentration 500 to 3000 ng/spot of RRB. Plate was developed using Toluene: Methanol: Triethanolamine (7:2.5: 0.5, v/v/v) at ambient temperature and dried in air. Developed plate was subjected to densitometric measurements in absorbance mode at wavelength 249 nm using TLC Scanner 3. A plot of peak area vs. concentration for drug was obtained. Spectra of drug were recorded in the range of 200-400 nm and purity of chromatographic peak was checked by scanning individual peak at 3 different positions (peak start, peak apex and peak end).

**Table 2: Linearity data for RRB**

| Conc. of Drug (ng/spot) | Area (Mean ± S.D) | C.V. |
|---|---|---|
| 500 | 2765.61 ± 29.07 | 1.05 |
| 1000 | 3350.64 ± 49.96 | 1.49 |
| 1500 | 3827.81 ± 13.18 | 0.33 |
| 2000 | 4385.3 ± 39.22 | 0.89 |
| 2500 | 4864.36 ± 70.93 | 1.45 |
| 3000 | 5495.6 ± 40.61 | 0.73 |

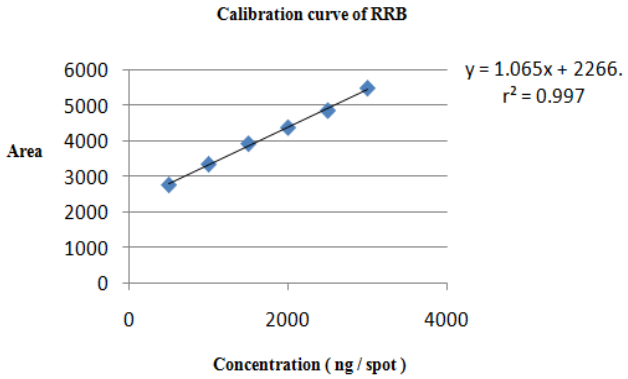


**Fig. 2: Calibration curve of RRB by HPTLC method**

**Accuracy studies (Recovery)**

Accuracy studies were performed by spiking test solution with standard solution. Accuracy studies were performed at spiking level of 50, 100 and 150% of target concentration. Here stock solution containing 500 µg/ml of RRB was prepared from tablet formulation. 2 µl of solution was applied from volumetric flask on to plate. Procedure was repeated for further 2 times and mean recovery for each level was calculated (n=3).

**Method Precision (Repeatability)**

For repeatability studies the linearity studies was repeated for 6 times without changing the syringe and position of plates. Data are collected from each set and Mean area, standard deviation and Coefficient of variance was calculated.

**Intra and inter day precision**

Variation of results within the same day (intra-day), variation of results between days (inter-day) was analyzed. Intraday precision was determined by analyzing concentration of 1000, 1500, 2000 ng/spot for RRB individually for three times in the same day. Inter-day precision was determined by analyzing same concentration of RRB individually for three days.

**Table 3: Accuracy data for RRB at 50, 100 and 150 % of target concentration**

| %Level of Recovery | Amount of drug in Sample (ng/spot) | Amount of Standard added (ng/spot) | Amount of drug recovered (ng/spot) | % Recovery ±SD |
|---|---|---|---|---|
| | RRB (ng/spot) | RRB (ng/spot) | RRB (ng/spot) | %RRB |
| Unspiked | 1000 | - | - | - |
| 50 % | 1000 | 500 | 506.78 ± 6.44 | 101.35 ± 1.28 |
| 100 % | 1000 | 1000 | 1011.89 ± 16.20 | 101.18 ± 1.61 |
| 150 % | 1000 | 1500 | 1511.22 ± 27.86 | 100.74 ± 1.85 |

**Table: 4 Precision studies for RRB by HPTLC method: (n=3 determinations)**

| Concentration (ng/spot) | Intraday (Area ± SD) | C.V. | Inter day (Area ± SD) | C.V. |
|---|---|---|---|---|
| **1000** | 3369.33 ± 13.01 | 0.38 | 3371.66 ± 15.27 | 0.45 |
| **1500** | 3936.96 ± 10.20 | 0.25 | 3943.93 ± 14.21 | 0.36 |
| **2000** | 4366.16 ± 15.91 | 0.36 | 4366.47 ± 20.81 | 0.47 |

**Table 5: Determination of LOD and LOQ**

| | Rivaroxaban |
|---|---|
| **LOD Based On mathematical Equation** | 127.56(ng/spot) |
| | **Rivaroxaban** |
| **LOQ Based On mathematical Equation** | 386.57(ng/spot) |

**Specificity Studies**

The excipients such as lactose, polyethylene glycol, and magnesium stearate were spiked into a reweighed quantity of drugs to assess the specificity of the methods. The peak area was measured to determine the quantity of the drugs.

**Robustness**

Robustness was performed by changing various method parameters like Composition of mobile phase, Size of TLC Chamber, Saturation time and plate pretreatment. Finally effect of these changes was observed for change in $R_f$ value and change in peak area. Spot stability was observed by performing 2-dimensional HPTLC development using the same mobile phase.

**Analysis of Marketed formulation**

Powder 10 tablets (Average weight of tablet 100mg), and take powder equivalent to10mg Rivaroxaban. Dissolve powder in 100 ml volumetric flask with 50 ml of Methanol. Sonicated for 15 minutes and make up volume up to mark with methanol. Filter above solution with whatmann filter paper (0.45 micron).Take1 ml of above solution and dilute up to 10 ml with methanol. From above solution apply 2 µl of volume on to pretreated Silica Gel F254 plates.

**Table 6: Robustness studies for HPTLC method**

| Parameter | Level of Change | Effect on Peak area | |
|---|---|---|---|
| | | RRB | |
| | | Area ± S.D. | % ASSAY± S.D. |
| Concentration of Mobile Phase | 6.8:2.3:0.2 | 3348 ± 1 | 101.59 ± 0.093 |
| | 7.2:2.7:0.7 | 3351.66 ± 1.5 | 101.94 ± 0.14 |
| Size of TLC chamber | 20*20 cm | 3353.66 ± 4.1 | 102.12 ± 39 |
| | 10*10 cm | 3361.33 ± 6.6 | 102.84 ± 62 |
| Saturation time | 20 min | 3362.33 ± 0.57 | 102.94 ± 0.054 |
| | 10 min | **Rf value is changed** | |
| Plate Pretreatment | Without Pretreatment | 3343. ± 7.21 | 101.12 ± 0.67 |

**Table 7: Assay of Marketed Formulation:**

| Formulation | Drug | Amount Taken (ng/spot) | Amount Found (ng/spot) (n = 3) | Labelled claim (mg) | Amount found per Tablet (mg) | % Label claim ± SD |
|---|---|---|---|---|---|---|
| RRB Tablet (Xarelto) | RRB | 1000 | 998.5 | 10 | 9.985 | 99.8 ± 1.45 |

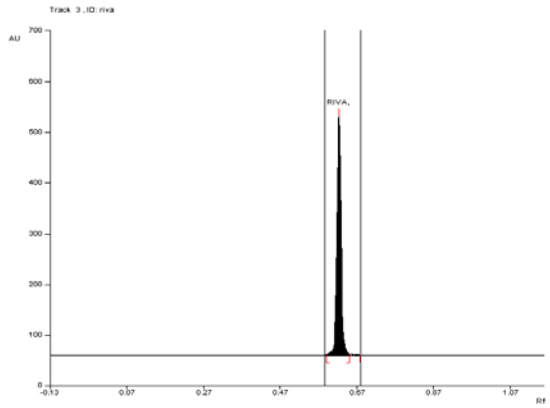


**Fig. 3: Densitogram of standard solution of RRB containing 1000 ng/spot using mobile phase Toluene: Methanol: Triethanolamine (7.0:2.5: 0.5, v/v/v)**

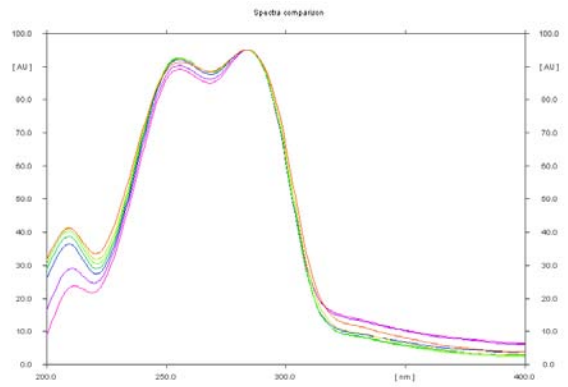


**Fig. 4: Overlay Absorbance spectrum of RRB (200-400 nm)**

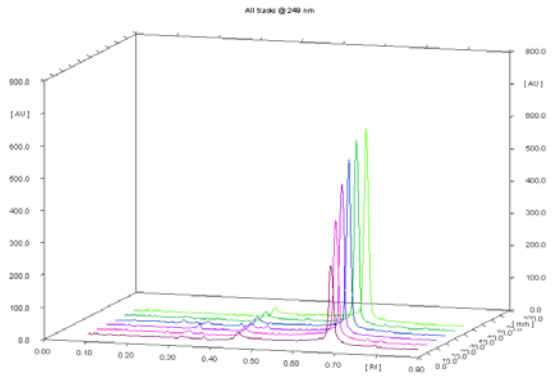


**Fig. 5: Overlay views of all tracks of RRB at 249nm**

## CONCLUSION

The HPTLC method was successfully developed and validated as per ICH guidelines and was successfully applied for rapid determination of Rivaroxaban from its tablet dosage form. The developed and validated HPTLC method for RRB was found to be simple specific and cost effective for analysis of RRB in its dosage form. The additives usually present in the pharmaceutical formulations of the assayed analytes did not interfere with determination of RRB. The method can be used for the routine analysis of RRB in pharmaceutical dosage form.

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