

QUANTIFICATION OF ANTICOAGULANTS DABIGATRAN, RIVAROXABAN, AND PRASUGREL BY CHROMATOGRAPHIC AND SPECTROMETRIC TECHNIQUES – REVIEW

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ABSTRACT

A review is presented on different analytical techniques used for quantitative analysis of selected anticoagulants dabigatran, rivaroxaban, and prasugrel. Efforts have been made to collate all the relevant references to the extent possible. The review discusses the advantages and disadvantages of the cited analytical techniques, which will help to give insights into the methods used for estimation of selected anticoagulants such as dabigatran, rivaroxaban, and prasugrel, from clinical isolates, and its dosage forms. The review highlights the basic as well as advanced techniques performed for estimating dabigatran, rivaroxaban, and prasugrel. The techniques illustrated here have been demonstrated to be useful for quantitative determination of selected anticoagulants and may find application in analyzing other related properties.

Keywords: Rivaroxaban, Dabigatran, Quantification, Prasugrel, Analyte.

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INTRODUCTION

A blood clot is a clump of blood that has changed from a liquid to a gel-like or semisolid state. Clotting or coagulation is a necessary process that can prevent you from losing too much blood in certain instances, such as when you're injured or cut. When a clot forms inside one of your veins, it won't always dissolve on its own. This can be a very dangerous and even life-threatening situation. There are many types of blood coagulation – deep vein thrombosis, pulmonary embolism, coronary embolism, and cerebral embolism. Hence, blood thinners are used (Anticoagulant), it is necessary to treat and prevent various blood clot *in vivo* condition.

In current scenario, numerous intravenous and oral anticoagulants are used for treatment of *in vivo* blood coagulation. Those are Indirect thrombin inhibitors-Heparin derivatives Fondaparinux, danaparoid, enoxaparin and dalteparin, Direct thrombin inhibitors- Lepirudin, Bivaluridin, Hirudin or Leechin and Oral ones- Coumarin derivatives- Nicoumalone, Warfarin, Indanedione derivatives- Phenindione and Diphendione, Director factor-Xa inhibitors-Apixaban, Rivaroxaban, Edoxaban, Platelet aggregator inhibitor-Clopidogrel, Prasugrel and Oral direct thrombin inhibitors-Dabigatran etexilate etc. [1].

Dabigatran is rapid-acting competitive and reversible direct inhibitor of thrombin. It is chemically as N-[2-[4-[N-(Hexyloxycarbonyl) amidino] phenylaminomethyl]-1-methyl-1H-benzimidazol-5-ylcarbonyl]-N-(2-pyridyl)-beta-alanine ethyl ester (Fig. 1). It is a light yellow to dark yellow solid with the empirical formula $C_{34}H_{41}N_7O_5$ and molar mass of 627.746 g/mol.

Rivaroxaban chemically described as 5-chloro-N-[[[5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl]-1, 3-oxazolidin-5-yl] methyl] thiophene-2-carboxamide (Fig. 2), it is a highly selective direct Factor Xa inhibitor. It is an empirical formula $C_{19}H_{18}ClN_3O_5S$ and molecular weight of 435.882 g/mol.

Prasugrel chemically prasugrel chemically is 5-[2-cyclopropyl -1-(2- fluorophenyl)-2-oxoethyl]-4, 5, 6, 7-tetra hydro thieno [3, 2- c] pyridin-2-yl acetate (Fig. 3), it reduces the aggregation (“clumping”) of platelets by irreversibly binding to P2Y12 receptors. It is empirical formula $C_{20}H_{20}FNO_3S$ and molecular weight of 435.882 g/mol.

In this article, we reviewed some analytical methods for the estimation of dabigatran, rivaroxaban, and prasugrel in pure drug and dosage forms.

QUANTITATIVE ANALYTICAL TECHNIQUES FOR SELECTED ANTICOAGULANTS

Analytical techniques for quantitative analysis help an analyst to accurately determine the concentration of an individual component in the test sample. The separation of the analyte is often performed before analysis by classical methods or during analysis by instrumental methods. Wet analytical methods such as titrations, precipitations, and extractions are used to estimate a drug or an analyte.

Instrumental methods for quantification of analyte use chromatography, electrophoresis, etc., for separation and physical properties such as absorption, fluorescence, conductivity, and light scattering are utilized to measure the analyte accurately and precisely.

The selected anticoagulants – dabigatran, rivaroxaban, and prasugrel can be estimated quantitatively using different methodologies. There are innumerable publications based on this simple basic technique, but a few were selected here.

Quantitative determination of dabigatran, rivaroxaban, and prasugrel**Spectroscopy**

It is impossible to envision any literature reference not taking advantage of this powerful technique in assaying dabigatran. Many studies have focused on this technique and have performed quantitative estimation of dabigatran as an active pharmaceutical ingredient or in its dosage form, either when formulated alone or with other active components.

Spectrophotometric method used for the analysis of tablets of dabigatran was cited by Jayavarapu *et al.* [2]. The method used 0.1 N hydrochloric acid as the solvent due to stability in HCl. The samples were scanned and analyzed at 326 nm wavelength. The method was found to be accurate, precise, and linear over the concentration ranges of 3–2 µg/mL. Similarly, Prajapati *et al.* [3] published spectrophotometric method for estimation of dabigatran etexilate in bulk and its pharmaceutical dosage form. Dabigatran etexilate was estimated at 313 nm. The linearity concentration ranges from 3 to 15 µg/mL. The method was carried out zero-order spectrophotometric method applied successfully for the analysis of the drug in the pure and pharmaceutical dosage form. Hepsebah *et al.* [4] developed

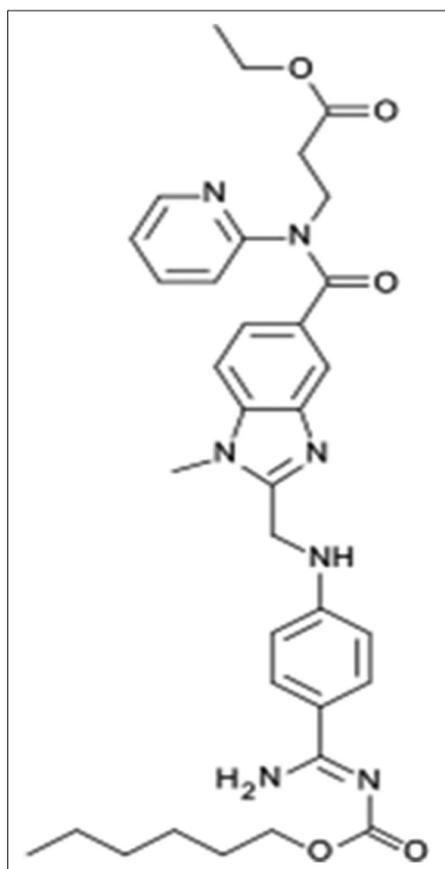


Fig. 1: Molecular structure of dabigatran

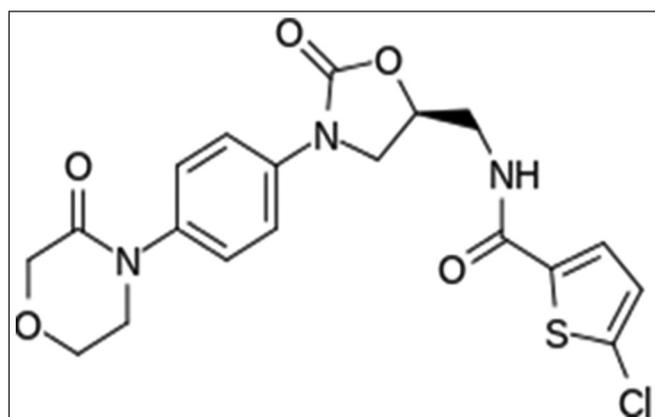


Fig. 2: Molecular structure of rivaroxaban

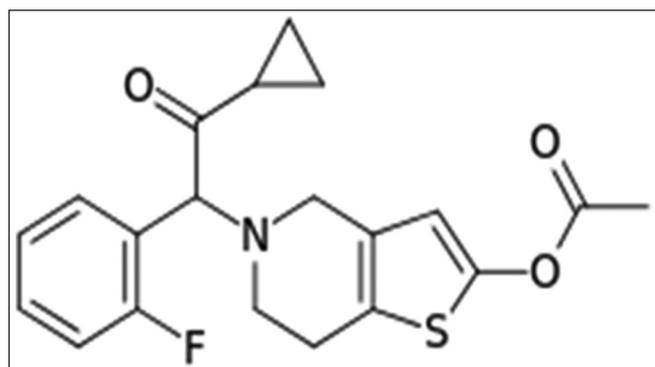


Fig. 3: Molecular structure of prasugre

spectrometric dissolution method for dabigatran etexilate mesylate capsules, using 0.01 N HCl as dissolution media, apparatus as USP Type 1 Basket, rpm as 100, dissolution media temperature as $37 \pm 0.5^\circ\text{C}$, dissolution volume as 500 ml, dissolution time point as 30 mts, and detection wavelength at 325 nm. The developed method resulted in dabigatran etexilate exhibiting linearity in the range 1.25–10 $\mu\text{g/ml}$. Harini *et al.* [5] developed and validated a simple, accurate, precise, and cost-effective UV-Spectrophotometric method for the estimation of dabigatran etexilate mesylate, the method was performed at 226 nm and the samples were prepared with methanol as solvent. Linearity range was found at 2–10 $\mu\text{g/ml}$ in UV with a correlation coefficient (R) 0.999. The proposed method was simple, precise, and accurate and can be used for the analysis of drug in bulk form. El-Din *et al.* [6] developed three rapid, accurate, and very simple derivative spectrophotometric methods for rivaroxaban and clopidogrel assay in their binary mixture and tablet dosage forms. First derivative spectrophotometric method, derivative amplitudes were measured at the zero crossing wavelength of 289 and 249.5 nm for estimation of rivaroxaban and clopidogrel, respectively. The linearity is over the range of 2.0–20.0 $\mu\text{g/ml}$ for rivaroxaban and 5.0–60.0 $\mu\text{g/ml}$ for clopidogrel. Seshamamba and Sekaran [7] described two simple spectrophotometric methods (I and II) for the determination of rivaroxaban in bulk and tablets. The method I is based on the reaction of rivaroxaban with 4-Chloro-7-nitrobenzo-2-oxa-1, 3-diazole in alkaline medium. Method II involved the reaction of rivaroxaban with p-Dimethylamino cinnamaldehyde in acidic medium. The reactions products were measured at 405 nm and 545 nm for methods I and II, respectively. Sekaran *et al.* [8] developed UV-spectrophotometric method for the quantification of rivaroxaban in bulk. Rivaroxaban was estimated at 270 nm in dimethyl sulfoxide. The linearity range was found to be 2–20 $\mu\text{g mL}^{-1}$. Sreeja *et al.* [9] developed two simple, sensitive, and economic spectrophotometric methods for the determination of desvenlafaxine in pure and its pharmaceutical formulations. In which proposed methods are based on the reaction of oxidative coupling of desvenlafaxine with 3-methyl-2-benzthiazolinonehydrazone (Method A) and 2, 2' bipyridyl (Method B) in the presence of ferric chloride. The colored complex produced was measured at 663 nm, 522 nm for method A and B, respectively, against the reagent blank. The optimum experimental parameters for the color productions are selected. Bagga *et al.* [10] developed that simple and cost-effective colorimetric method is described for the determination of prasugrel in pure form and pharmaceutical formulations. The determination of the drug in pharmaceutical formulations is based on the drugs redox reaction with Folin-Ciocalteu reagent (Phenol reagent). The linearity range for prasugrel of blue chromogen produced at a wavelength of detection 725 nm was obtained as 10–50 $\mu\text{g/ml}$. The absorbance was found to increase linearly with increasing concentration of prasugrel, which is corroborated by the calculated correlation coefficient value of 0.999. Another simple, economical, precise, and reliable spectrophotometric method was done by Harshini *et al.* [11] for the estimation of prasugrel. In this method, the formation of chloroform extractable complex of prasugrel with bromocresol green this shows absorbance maximum at 418 nm. Sowndarya *et al.* [12] developed a simple and cheap UV-spectrophotometric method for the quantitative estimation of dabigatrn etexilate (150 mg) in capsules and the optimized method uses 0.05 N HCl as a solvent for the estimation of an assay of dabigatrn etexilate in capsules at a wavelength of 325 nm. The developed method resulted in dabigatrn etexilate exhibiting linearity in the range 5–15 $\mu\text{g/ml}$. The assay precision is exemplified by the relative standard deviation of 1.4%. Seshamamba and Sekaran [13] studied and revealed the report on the development of two spectrophotometric methods (A and B), for the determination of rivaroxaban in bulk and Xarelto tablets. Method A involves the formation of colored tris (1, 10-phenanthroline)-iron (II) complex, on reaction of rivaroxaban with iron (III)-1, and 10-phenanthroline mixture in acidic media. The colored complex is spectrophotometrically measured at 525 nm. Method B involves the interaction of rivaroxaban with acetaldehyde with chloranil, to give colored vinyl amino substituted quinones. The colored product exhibit absorption maxima at 655 nm. Beer's law correlating the absorbance with rivaroxaban concentration was obeyed

in the range of 5–62.5 µg/ml and 10–100 µg/ml, for methods A and B, respectively. Alvi *et al.* [14] developed two simple, accurate, and precise UV derivative spectrophotometric methods for the simultaneous determination of prasugrel and aspirin in synthetic mixture form. The first method involves the measurement of second-order derivative spectra of prasugrel and aspirin. The zero crossing wavelengths 267.62 nm and 252.40 nm were selected for estimation of prasugrel and aspirin, respectively. In the second method, the first-order derivatives of ratio spectra were calculated and used for the determination of prasugrel and aspirin by measuring the peak intensity at 268 nm and 290 nm, respectively. Çelebier *et al.* [15] studied that a UV-spectrophotometric method was developed for the determination of rivaroxaban content in pharmaceutical formulations and the amount of rivaroxaban released in tablet dissolution studies. The dissolution profile of rivaroxaban was successfully determined with the validated method. Sharaf *et al.* [16] created three rapid, accurate, and very simple derivative spectrophotometric methods for rivaroxaban and clopidogrel assay in their binary mixture and tablet dosage forms were developed. Method (I) is the first derivative spectrophotometric method; derivative amplitudes were measured at the zero crossing wavelength of 289 and 249.5 nm for estimation of rivaroxaban and clopidogrel, respectively. Method (II) is a ratio derivative spectrophotometric method. The ratio spectra of each drug were derived by dividing its spectra on a constant concentration of the other drug as a divisor. Derivative amplitudes were measured at 256 nm for rivaroxaban and at 214.5 nm for clopidogrel and in Method (III) is absorbance ratio method, the absorbance of both drugs was recorded at two wavelengths λ_1 (232) iso-absorptive point and λ_2 (249) λ_{max} of rivaroxaban and clopidogrel. Rajendiran *et al.* [16] developed new analytical methods such as UV Spectroscopy and reversed-phase high-performance liquid chromatographic (RP-HPLC) for the estimation of prasugrel hydrochloride in bulk and tablet dosage form. In the spectroscopic method, prasugrel hydrochloride showed maximum absorbance at 252 nm in methanol which is selected as a solvent for analysis based on its stability. Beer's law obeyed in the concentration range of 10–60 mcg/ml. In RP-HPLC, the mobile phase selected is acetonitrile: trifluoroacetic acid in the ratio 45:55% v/v. The flow rate was 0.1 ml/min. The linearity range was found to be 10–60 mcg/ml. The formulation EF1PLT was selected for analysis and the amount present was found to be 100.53% and 106.95% for method UV and RP-HPLC, respectively. Mehta and Maheshwari [17] developed UV-spectrophotometric methods and LC assay methods and validated for the determination of rivaroxaban and ticagrelor in the synthetic mixture. Spectrophotometric estimation was done by derivative spectroscopic method and methanol as solvent. In this method, λ_{max} for rivaroxaban and ticagrelor were selected at 295 nm and 249 nm. RP-HPLC analysis was carried out using Pearless C-18 column (4.6×250 mm, 5 µ particle size) and mobile phase composed of acetonitrile:10% orthophosphoric acid in water pH 4.0 (60:40% v/v) at a flow rate of 1.0 ml/min and chromatogram was recorded at 249 nm.

Chromatography

Today not a single molecule is analyzed without the use of powerful analytical tools such as HPLC, ultra-performance LC (UPLC), and high-performance thin-layer chromatography (HPTLC).

Geetharam *et al.* [18] developed simple, precise, accurate, and rapid stability indicating that HPLC method was developed and validated for the determination of dabigatran etexilate in bulk and capsule dosage forms. Chromatographic separation was achieved using mobile phase phosphate buffer Ph 4.5 and acetonitrile in the ratio 50:50 with hypersil BDS C₁₈ (150×4.6 mm, 5 µm) as stationary phase. Flow rate was optimized to 1.2 ml/min and effluents were monitored at 230 nm. The retention time was around 3.19. Linearity was observed over a concentration range of 9–113 µg/ml with correlation coefficient of 0.999. Abdallah *et al.* [19] developed and validated an orthogonal stability-indicating testing protocol (RP-HPLC and NP-TLC-densitometry) for the quantification of Rivaroxaban. It is an amide group-containing oral anticoagulant was subjected to stress conditions commonly required for the registration of pharmaceuticals: base and

acidcatalyzed hydrolysis (0.1 M, 60°C, 3–6 h), oxidation (10% H₂O₂, 24 h), photodegradation (300–800 nm, 24 h) and thermal decomposition (50°C, 6 h). Two major degradation products were separated and identified using TLC and LC/MS/MS, respectively. The Determination was carried out over a concentration range of (5.00–50.00 µg/mL) and (0.40–12.00 µg/band) with an accuracy of (100.81% ± 1.03) and (100.29% ± 1.08) for HPLC and TLC-densitometry, respectively. Nagadeep *et al.* [20] developed and validated RP-HPLC method for separation and determination of potential related impurities (starting materials and by-products of synthesis, and degradants) of dabigatran etexilate drug substance. The separation was accomplished on an Inertsil ODS 3V (250 mm×4.6 mm, 5 µm) column connected to a photodiode array (PDA) detector using 20 mM ammonium formate with 0.1% of triethylamine (TEA) (pH: 5.0 adjusted with formic acid) as mobile phase-A and acetonitrile as mobile phase-B, under gradient elution. Kumar *et al.* [21] developed a RP-HPLC bioanalytical method development and validation for prasugrel in human plasma using PDA detector. The HPLC separation was carried out in an isocratic mode on an X-Terra C18 column (4.6×150 mm; 5 µm) with a mobile phase consisting of potassium dihydrogen phosphate [pH 3.0] and acetonitrile in the ratio of 30:70 v/v at a flow rate of 1.0 mL/min. The run time was maintained for 5 min and the detection was monitored at 210 nm. Damle and Bagwe [22] developed and validated a simple, selective, and rapid stability indicating RP-HPLC method for the estimation of dabigatran etexilate analyte and it was resolved on a Neosphere C8 (150 mm×4.6 mm) column. The mobile phase consisted of methanol: phosphate Buffer (0.01 M pH 3) in the ratio of 60:40 v/v and sonicated to degas. It was delivered at a flow rate of 1 ml/min at ambient temperature and the retention time was about 4.4±0.05 min. Studies were performed on an HPLC system equipped with a PDA detector at 225 nm. Ishaq *et al.* [23] designed to develop and validate a simple, sensitive, precise, and specific RP-HPLC method for the determination of prasugrel in bulk and its tablet dosage forms. Separation was carried out by RP chromatography on an inertsil ODS-3V column (5 µm; 250×4.6 mm²) with a mobile phase composed of 0.02 M potassium dihydrogen orthophosphate, 0.02 M dipotassium hydrogen orthophosphate in water: acetonitrile (30:70 v/v) in isocratic mode at a flow rate of 1 ml/min. The detection was monitored at 210 nm. Sagar *et al.* [24] developed a rapid, simple, precise, accurate, and reproducible RP-HPLC method for simultaneous determination of aspirin and prasugrel in the tablet dosage form. The estimation was carried out on a HIBAR (Lichrospher C18) column with the dimensions of 250 mm×4.6 mm, 5 µm. Combination of acetonitrile and 0.5% potassium dihydrogen phosphate buffer (adjusted to pH-3 using orthophosphoric acid) in the ratio of 60:40 was used as mobile phase. The flow rate is set at 1.0 ml/min and eluents were monitored at 220 nm. Sahoo and Mekap [25] optimized a RP-HPLC procedure to finalize a different approach for the estimation of rivaroxaban in tablet dosage form. The determination was executed by C₁₈ column (Phenomenex 250×4.6 mm, 5 µm maintained at 35°C) at 251 nm with a mobile phase (ACN: Water, 55:45 v/v) and flow of 1.2 ml/min. The retention time found to be about 3.8 min. Manasa *et al.* [26] developed a rapid, sensitive, accurate, precise, and linear stability indicating RP-HPLC assay method and validate as per the ICH guidelines for the estimation of dabigatran etexilate mesylate in capsules. The optimized method employs a RP column, Phenomenex Kinetex EVO C18 (250×4.6 mm; 5µ), a mobile phase of triethylammonium phosphate buffer (pH 2.0):methanol: acetonitrile in the proportion of 30:30:40 v/v, flow rate of 0.6 ml/min and a detection wavelength of 254 nm using a UV detector. Souri *et al.* [27] studied a stability-indicating HPLC method for the quantitative determination of rivaroxaban in pharmaceutical dosage forms. Rivaroxaban was subjected to acidic, basic, oxidative, photolytic, and thermal conditions for forced stress degradation studies. Considerable degradation was observed in all stress degradation tests. Rivaroxaban and its degradation products were separated on a Nova-Pak C8 column utilizing a mixture of acetonitrile and KH₂PO₄ 50 mM (pH 3.0) (40:60, v/v) as the mobile phase, and the chromatogram was recorded at 270 nm using a general ultraviolet detector. Seshamamba *et al.* [28] developed simple and sensitive stability indicating HPLC method for

the quantification of rivaroxaban in bulk and tablet dosage form. Chromatographic separation was achieved on an ACE-Ciano column (250 mm×4.6 mm, 5 µm particle size). The mobile phase consists of 0.1 M sodium acetate and methanol (60:40 v/v) and was delivered at a flow rate of 1 mL/min. A UV detector was used for detection. Reddy *et al.* [29] developed and validate RP-HPLC, simple, precise, accurate, and specific chromatographic method for the determination of related substances of prasugrel in pharmaceutical formulations. HPLC instrument and Inert Sustain C18, 75×4.6 mm, 3 µm were used for determination of prasugrel and its related substances. The buffer was prepared using 1.36 g of potassium phosphate monobasic in 1000 mL of water, adjust the pH of this solution to 3.30 with a dilute orthophosphoric acid solution and mix well. Filter through 0.45 µm nylon filter and degas. The buffer used as a mobile phase-A, the mobile phase-B was prepared by mixing acetonitrile and water in the ratio of 90:10 (v/v). The flow rate of 1.0 mL/min was set with gradient program, the temperature of the column compartment maintained at 30°C and ultraviolet detection done at 235 nm wavelength. Nawale *et al.* [30] developed and validate new, simple, and selective RP-HPLC method for the quantitative determination of dabigatran etexilate and its impurities in the pharmaceutical dosage form as per the ICH guidelines. Chromatographic analysis was performed on Princeton SPHER-100 C18 (250×4.6 mm, 5 µm) HPLC column, maintained at 50°C column temperatures, 6°C sample tray temperature, and detection monitored at 225 nm. The mobile phase consisted of acetonitrile: phosphate buffer (pH 2.5) (33:67 V/V). The flow rate was maintained at 1.0 ml/min. Bhavani *et al.* [31] developing simple, fast, and effective dissolution method for dabigatran etexilate mesylate capsules by RP-HPLC and validate as per the ICH guidelines. The optimized RP-HPLC method for dissolution studies uses a RP column, Phenomenex Luna C18 (250×4.6 mm; 5µ), a mobile phase of triethylammonium phosphate buffer (pH 3.0):acetonitrile in the proportion of 40:60 v/v, diluent as 0.01N HCl, flow rate of 1.0 ml/min, injection volume as 20 µl, and a detection wavelength of 341 nm using a UV detector. Balaji *et al.* [32] developed a validated, simple, precise, and stability indicating RP-HPLC method for the determination of dabigatran etexilate mesylate in bulk drug and dosage form. LC separation was achieved gradient mode on a Zorbax SB C₁₈ (4.6×150) mm, 3.5 µm column using mobile phase containing solution A (2.72g of potassium dihydrogen phosphate in 1000 ml of water) PH 4.5 with orthophosphoric acid solution B (acetonitrile) at flow rate 1.0 ml/min. The method employed a linear gradient elution and detection wavelength was set at 220 nm. Bernardi *et al.* [33] developed and validated an RP-LC method for the determination of dabigatran etexilate in a capsule formulation. The LC method was carried out on a Zorbax C18 column (250×4.6 mm id). The mobile phase consisted of acetonitrile and a solution of TEA 0.1%, pH 6.0 adjusted with phosphoric acid (65 + 35, v/v) at a flow rate of 1.0 mL/min. The diode array detector was set at 225 nm. The chromatographic separation was obtained with a retention time of 6.31 min. Patel *et al.* [34] a simple and precise stability indicating that HPTLC method has been developed and validated for the simultaneous estimation of prasugrel and aspirin from dosage form. The method employed TLC aluminum plates precoated with silica gel 60 GF 254 as the stationary phase. The solvent system comprised carbon tetrachloride: ethyl acetate: acetic acid (7.5:2.4:0.1, v/v/v). This system was found to give good result for both the drugs (Rf value: of aspirin 0.34 cm and Prasugrel 0.58 cm). Spectrodensitometric scanning-integration was performed at a wavelength of 240 nm. Avachat and Yamgar [35] developed and validated a RP-HPLC method for the estimation of rivaroxaban in bulk and tablet dosage forms. The separation was achieved on stainless steel Purospher® STAR Hibar® C18 analytical column (250 mm×4.6 mm i.d., 5.0 µm) using acetonitrile and water in the ratio 80:20 v/v as mobile phase and at a flow rate of 1 mL/min. Detection was carried out using a UV detector at 247 nm. Liu *et al.* [36] detected a brand-new impurity by RP-HPLC in the prasugrel. The impurity was named as impurity X. Impurity X was isolated using semi-preparative HPLC followed by characterization using nuclear magnetic resonance spectroscopy and LC-MS. The functional mechanism of impurity X was speculated. Impurity X could be controlled in the manufacture process of the

prasugrel active pharmaceutical ingredient effectively. BRC Sekhar Reddy and Nallagatla. Vijaya Bhaskar Rao. Reddy and Rao [37] validated that RP-HPLC is used for the estimation of dabigatran in bulk and pharmaceutical dosage form. It is a simple, precise, accurate RP-LC. The HPLC method has been carried out by using Chromasil C18 column. This method has been developed using the mobile phase consisting methanol: acetonitrile:water 80:15:5 (v/v). pH 4.7 at the flow rate of 1.0 ml/min by the detection of UV at 280 nm. The retention time of the dabigatran is 5.73 min. Ishaq *et al.* [38] developed and validated a simple, sensitive, precise, and specific RP-HPLC method for the determination of prasugrel in bulk and tablet dosage forms. The HPLC separation was carried out by RP chromatography on inertsil ODS-3V column (5 µm; 250×4.6 mm) with a mobile phase composed of 0.02 M potassium dihydrogen orthophosphate, 0.02 M dipotassium hydrogen orthophosphate in water: acetonitrile (30:70 v/v) in isocratic mode at a flow rate of 1 ml/min. The detection was monitored at 210 nm. Parmar *et al.* [39] developed RP-LC method for the assay of prasugrel in bulk. The chromatography was performed on Kromasil C18 column. The eluted compounds were monitored by UV detection at 257 nm using mobile phase methanol-potassium dihydrogen orthophosphate (pH 2.2; 10 mM) (70:30, v/v). Hadagali [40] developed and validated a simple, sensitive, and rugged RP-HPLC method for the determination of rivaroxaban in pure, pharmaceutical formulations, and in spiked human plasma sample. The separation of rivaroxaban and internal standard was achieved on XDB C18 (150×4.6) mm column. Mobile phase employed for the study is the mixture of water and acetonitrile in gradient program. A flow rate of 1 ml/min was found optimum for the study. Srinivas *et al.* [41] developed a simple, precise, accurate, economical, and reproducible RP-HPLC method for estimation of dabigatran etexilate mesylate in capsule dosage form. Quantitative HPLC was performed with Shimadzu LC 20AT with Spin chrome Software with UV-Visible Detector (SPD-20A), Phenomenex Luna C18, 5µm, 250×4.6 mm (size) column was used in the study. The mobile phase of methanol: eater (70:30) used in this study. The conditions optimized were flow rate (1.2 ml/min) and wavelength (230 nm) and run time was 10 min, column temperature was maintained at 500 C. Retention time was found to be 4.60 min. Sonia *et al.* [42] developed and validated of prasugrel hydrochloride 5 mg tablet by RP-HPLC for precision, accuracy linearity, and robustness. Zorbax, SB-Phenyl 250×4.6 mm, 5µ in isocratic mode, with mobile phase containing buffer (pH 6.5 with TEA and potassium dihydrogen orthophosphate) acetonitrile: eater (90:10) [40–60 (v/v)] was used. The flow rate was 1.2 ml/min and the analyte was monitored at 235 nm. The retention time for prasugrel HCL was 11.5 min. Dalmora *et al.* [43] a stability indicating that RP-HPLC method was validated for the analysis of rivaroxaban. The method was carried out on a C18 column (150 mm×4.6 mm i.d.), maintained at 40°C. The mobile phase consisted of acetonitrile and water (70:30), run isocratically at a flow rate of 0.7 mL/min, and using a PDA detection at 249 nm. The separation was obtained with retention time of 2.9 min. Prajapati and Patel [44] described a precise, accurate, and reproducible RP-HPLC method for simultaneous estimation of clopidogrel and rivaroxaban. The separation was carried by Kromasil C18 column (250×4.6 mm, 5 µ) with UV detection at 240 nm. The mobile phase is consisting of acetonitrile and water in a ratio of 90:10, v/v and at a flow rate of 1 mL/min. The method was linear over the concentration range for clopidogrel 1.0–20.0 µg/mL and for rivaroxaban 1.0–20.0 µg/mL. Sriram *et al.* [45] described the development of RP-HPLC method for prasugrel hydrochloride in the presence of impurities and degradation products generated from the forced degradation studies. Successful separation of the drug from the process related impurities and degradation products were achieved on Gemini C18 (250×4.6 mm) 5 µm particle size column using RP-HPLC method. The isocratic method employed with a mixture of buffer and (10%v/v water in acetonitrile) mixture of ratio 30:70, respectively. Potassium dihydrogen orthophosphate (0.05 M) is used as buffer. Kasad [46] developed and validated a simple, precise, and accurate RP-HPLC method for assay of rivaroxaban. An isocratic separation was achieved using a phenomenex C18 (250×4.6 mm, 5 µm), 100Å particle size columns with a flow rate of 1 ml/min and using a PDA detector to

monitor the elute at 250 nm. The mobile phase consisted of methanol: acetonitrile (50:50 v/v). Raja *et al.* [47] developed and validated a simple, rapid, and reproducible RP-HPLC method for the estimation of prasugrel hydrochloride in tablet dosage form. A Zorbax, SB-Phenyl 250×4.6 mm, 5 μ in isocratic mode, with mobile phase containing buffer (pH 6.5 with TEA and potassium dihydrogen orthophosphate):acetonitrile: water (90:10) [40:60 (v/v)] was used. The flow rate was 1.2 ml/min and the analyte was monitored at 235 nm. The retention time for prasugrel HCl was 11.5 min. Rigobello *et al.* [48] a stability indicating that RP-LC method for the determination of prasugrel in tablets was developed and validated. Stress testing of prasugrel was carried out in accordance with ICH guidelines, where the drug was submitted to acidic and basic hydrolysis, oxidative, thermal, and photolytic conditions. Prasugrel was unstable under all the conditions and the degradation products were analyzed by HPLC-UV. Furthermore, two main degradation products found under alkaline and thermal conditions were investigated by LC-MS. Based on the fragmentation patterns, two products resulted from hydrolysis of the acetate ester moiety of prasugrel were observed. Due the chemical equilibrium, tautomerism occurs between the ketone and alcohol functions justifying the similar molecular weight and fragment pattern obtained in degradation products analysis. Successful separation was achieved on a RP-18 octadecylsilane column using acetonitrile and TEA 0.5% mixture (50:50, v/v) as the mobile phase at 25°C. The flow rate was 1.0 mL/min and the detector wavelength was 263 nm. Ahirrao *et al.* [49] developed a simple, rapid, and precise method for the quantitative estimation of prasugrel hydrochloride in pharmaceutical dosage form. A chromatographic separation of prasugrel and its degradants was achieved with Zorbax XDB C8, 150×4.6 mm, 3.5 μ m analytical column using aqueous solution of 0.05 M ammonium acetate pH 4.5 with acetic acid-acetonitrile (40:60 v/v). The instrumental settings include flow rate of 1.0 ml/min, column temperature at 30°C, and detector wavelength of 254 nm using a PDA detector. Modi and Pingale [50] developed and validated a new, simple, specific, accurate, precise, and rapid RP-HPLC method for the determination of prasugrel hydrochloride in pure and tablet dosage forms. The HPLC separation was carried out by RP chromatography on Kromasil C18 (100×4.6 mm; 5 μ m) with a mobile phase consist of methanol buffer (680 mg potassium dihydrogen phosphate in 500 ml water, pH-2.1 adjusted with orthophosphoric acid) in the ratio of 70:30 v/v delivered in isocratic mode at a flow rate of 0.8 ml/min. The prasugrel hydrochloride was quantified at 220 nm. The retention time of prasugrel hydrochloride was 1.9 min. Kumar *et al.* [51] designed a simple, sensitive, precise, and specific RP-HPLC method for the determination of prasugrel in bulk and its tablet dosage forms. The HPLC separation was carried out by RP chromatography on XTerra column C18 (4.6×150 mm, 5 μ m) with a mobile phase composed potassium dihydrogen phosphate and the pH has been adjusted to 3.0 by Orthophosphoric acid and acetonitrile in the ration of 40:60 v/v in isocratic mode at a flow rate of 1.0 ml/min. The run time has been maintained 5 min. The detection was monitored at 210 nm. Shivashankar *et al.* [52] developed and validated a simple RP-HPLC method for the estimation of rivaroxaban in formulation. A HIBAR-5 μ C18 column (250×4.6 mm) was used as a stationary phase. The mobile phase consisted of potassium dihydrogen orthophosphate buffer (pH adjusted to 3.0 with orthophosphoric acid):acetonitrile in the ratio of 60:40 % v/v and the flow rate was 1 ml/min. The detection was carried in the room temperature at 248 nm. The retention time of rivaroxaban was 7.45 min. Latha *et al.* [53] developed and validated a RP-HPLC method for the determination of dabigatran etexilate mesylate (DEM) in bulk form. The chromatography was carried out on Unisol C18 (4.6×150 mm, 3 μ m) using a mobile phase of methanol and ammonium acetate buffer in the ratio of 90:10, at a flow rate of 1 ml/min. The analytes were monitored at 226 nm using a PDA detector. The retention time of the DEM was 2.52 min. Dare *et al.* [54] developed the stability indicating, highly accurate precise and linear method for related substance of the dabigatran through the RP-HPLC. The optimize method uses a RP column, Poroshell 120 EC-18 (150 mm×4.6 mm, 2.7 μ), mobile phase of hexane-1 sulfonic acid sodium salt monohydrate (6.5±0.05), and methanol through gradient flow rate of 0.6 ml/min.

Keeping the column at temperature 30°C and using the sample amount 10 μ L at 5°C and detected all the impurities at 230 nm by the UV detector. In the developed method, elution of dabigatran was at 26.9 min. El Zaher *et al.* [55] carried a simple, precise, accurate, and rapid stability indicating that LC method was developed for the determination of dabigatran etexilate mesylate in bulk powder and pharmaceutical formulations. Chromatographic separation was carried out by isocratic elution on Eclipse XDB C8 (4.6×250 mm, 5 μ m) using 0.01 M orthophosphoric acid (pH 2.6):acetonitrile (60:40 v/v) as the mobile phase. The flow rate was 1.5 ml/min and the UV detection was done at 225 nm. Linearity was obtained over a concentration range of 5–100 μ g/ml with a regression coefficient of 0.9975. Patel *et al.* [56] developed and validated an accurate, sensitive and rapid RP-HPLC method for the estimation of dabigatran etexilate mesylate from capsule dosage form. The separation was achieved by a Zorbax C18 column (100 mm×4.6 mm, 3.5 μ m) in isocratic mode, with mobile phase comprises of acetonitrile: water in the proportion of 70:30 v/v. The flow rate of mobile phase was 1.0 ml/min, and detection was done at 225 nm wavelength. The retention time of dabigatran etexilate mesylate was 3.0 min. The calibration curve was found to be linear with in the concentration range of 20 μ g/ml–100 μ g/ml. Çelebier *et al.* [57] developed a RP-HPLC method for the determination of rivaroxaban in tablets (Xarelto® [10 mg]). Phenomenex Luna 5 μ m C18 100 Å LC Column (250×4.6 mm) was used at 40°C. Isocratic elution was performed with ACN: water (55:45 v/v) mixture. The flow rate was 1.2 mL/min and UV detection was at 249 nm. Internal standard (Caffeine) and rivaroxaban were eluted within 2.21 and 3.37 min, respectively. Girase and Soni [58] developed and validated RP-HPLC method for the qualitative analysis of rivaroxaban. The chromatographic separation was achieved on ZorbaxSB C18 (250 mm×4.6 mm, 3.5 μ) HPLC column using buffer (0.02M mono basic potassium dihydrogen phosphate) and solvent mixture (acetonitrile: methanol mixture) ingradient program. Rajan and Basha [59] developed and evaluated the RP-UPLC method for the quantitative determination of potential impurities of rivaroxaban active pharmaceutical ingredient. The method uses a water acquity BEH C8 column (100 mm×2.1 mm, 1.7 μ m) with mobile phase A consisted, 0.05M diammonium hydrogen phosphate, pH adjusted to 3.0 and acetonitrile (80:20, v/v), and mobile phase B consisted mixture of acetonitrile and water (90:10, v/v) with a gradient program. The column temperature was maintained at 30°C and the detection was carried out at 254 nm. El-Aziz *et al.* [60] created simultaneous assay method of two anti-platelet drugs, rivaroxaban with clopidogrel the developed and validated method is characterized by its simplicity, sensitivity, and rapidness. Chromatographic study is performed on a 3 mm×4.6 mm i.d., 2 μ m pore size highly porous monolithic (Chromolith® RP) column to obtain good chromatographic separation using a mixture of 0.185 M sodium dodecylsulfate, 8% n-propanol, 0.3% TEA as a mobile phase, and adjusted at pH 4.0 using 0.02 M orthophosphoric acid and pumped at a flow rate of 1 mL/min using UV detector at 235 nm. The internal standard (IS) of choice was raloxifen and separation was performed at room temperature. Good linearity for the proposed method was obtained over the ranges of 0.3–9.0 μ g/mL and 0.5–25.0 μ g/mL and retention time was 1.9 and 5min, respectively. Meenakshi and Rao [61] developed that simple, rapid, accurate, precise, robust, and reproducible RP-HPLC method for the determination of rivaroxaban in pure drug and pharmaceutical dosage form. The quantification was carried out using enable C₁₈ 250×4.6 mm, 5 μ m) column in a binary mode with mobile phase comprising 0.1% glacial acetic acid: acetonitrile in 30:70% v/v at flow rate 1 ml/min, detection was carried out at 250 nm using PDA detector with injection volume 20 μ l, the retention time was found to be 3.44 min. Sahani and Jain [62] developed and validate a simple, rapid, accurate, and precise RP-HPLC method for simultaneous estimation of drotaverine hydrochloride, ethamsylate, and tranexamic acid in the tablet dosage form. Sunny *et al.* [63] developed RP-HPLC method for the quantitative estimation of rivaroxabanin formulation and bulk drug. Isocratic elution at a flow rate of 1 ml/min was employed on an Agilent Eclipse C8 LC Column (150×4.6 mm) with 5 μ m pore size was used at 40°C. The mobile phase consisted of TEA: acetonitrile 85:15 v/v (P^H

11.6). The UV detection wavelength was at 251 nm and 20 μ l, the sample was injected at an interval of 5 min. Retention time for rivaroxaban was 1.56 min. Prajapati *et al.* [64] carried stability-indicating HPTLC method for the estimation of dabigatran etexilate mesylate using TLC plates pre-coated with silica gel 60 F254 as stationary phase and toluene: ethyl acetate: methanol:formic acid (3:4:3:0.2, v/v/v/v) as the mobile phase. The wavelength selected for quantitation was 314 nm. The method was linear in the concentration range 50–250 ng/spot with a correlation coefficient of 0.9955. Vaghela and Patel [65] developed and validated HPTLC, simple, precise, accurate, and specific chromatographic method for the determination of rivaroxaban from its tablet dosage form. The mentioned drug was spotted on silica gel F254 TLC plates under pure nitrogen stream by Linomat TLC spotter. Separation was carried out 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. Rf value of separated drug was found to be 0.60 for rivaroxaban. The linearity of rivaroxaban is 500–3000 ng/spot. Srikanth *et al.* [66] proposed that simple, sensitive and precise RP-HPLC method has been developed for the estimation of prasugrel hydrochloride in pharmaceutical dosage forms. The mobile phase consist of buffer (0.02 M sodium dihydrogen phosphate, pH-3 adjusted with orthophosphoric acid):methanol: acetonitrile in the ratio of 45:20:35v/v delivered at a flow rate of 1.0 ml/min and wavelength of detection at 220 nm. The retention time of prasugrel hydrochloride was 8.2 min. Abu Ruzilawati and Miran [67] discussed and developed that methamphetamine is one of the illicit drugs, which are common among students and teenagers. Methamphetamine was found to be the most commonly abused amphetamine-type stimulants compared with other types of amphetamines. Therefore, fast and reliable detection of these drugs is important in toxicology and doping control. Many extraction methods such as solid phase (SPE) and SPE microextraction have been used for extraction of methamphetamine in the biological fluid. The objective of this study was to develop an effective liquid-liquid extraction method from methamphetamine in human urine. Optimization of the extraction method was done by varying the types of solvents used to extract the drug from the urine. The percentage recovery of methamphetamine extraction was investigated using a variety of extracting solvents (ethyl acetate, dichloromethane, diethyl ether, and chloroform) and solvents system (chloroform: isopropanol [9:1] and chloroform: ethyl acetate: ethanol [3:1:1]). The analysis of the extracted drug was done by HPLC equipped with fluorescence detector. Optimization of chromatographic conditions was also done by varying the pH's of mobile phase and the composition of the mobile phase. The extracting solvent system that consists of chloroform: ethyl acetate: ethanol (3:1:1) gave the highest recovery, which is 87%. The optimized HPLC condition used for the analysis was a mixture of acetonitrile and deionized water at pH 2.4 in the ratio of 15:85 v/v with a flow rate of 0.6 mL/min.

LC-MS

Antovic *et al.* [68] carried that dabigatran concentration in plasma was determined using LC-MS/MS. Chromatographic separation of the analytes was achieved on an Acquity UPLC BEH column (Shield RP₁₈, 2.1×50 mm, 1.7 μ m) using a gradient run with mobile phase A (10 mM ammonium formate pH 4.5) and mobile phase B (0.1% formic acid in acetonitrile). The analytes were detected using a Waters Quattro Premier XE mass spectrometer operating in positive electrospray ionization (ESI) mode, utilizing selected reaction monitoring (SRM) with ion transitions 472→289 m/z for dabigatran and 475→292 m/z for the internal standard. The total analysis time was 3 min. Srikanth and Rani [69] developed and validate a LC-MS/MS for the quantification of lenvatinib (LT) in human plasma. A simple, sensitive, and specific LC-MS/MS method was developed for quantification of LT in human plasma using LTD4 as internal standard (IS). The analytical method consists of liquid-liquid extraction of plasma sample followed by the determination of LT by an LC-MS/MS. The analyte was separated on a Zorbax Eclipse XDB-C18 (150×4.6 mm, 5 μ) column with an isocratic mobile phase of acetonitrile:0.1% formic acid (80:20 v/v) at a flow rate

of 0.6 mL/min. The product ions were formed by a turbo ion spray in a positive mode were used to detect analyte and IS. The MS/MS detection was made by monitoring the fragmentation of m/z 427.10→370.10 for LT and m/z 430.30→370.10 for IS on a MS. Schmitz *et al.* [70] carried out determination of dabigatran, rivaroxaban, and apixaban by UPLC-MS/MS and coagulation assays for therapy monitoring of novel direct oral anticoagulants. Zhang *et al.* [71] established a rapid and sensitive UPLC-MS/MS method for the determination of rivaroxaban, apixaban, and edoxaban in rat plasma. The analytes and the internal standard (diazepam) were separated on an Acquity UPLC BEH C₁₈ chromatography column (2.1 mm, 9.50 mm, 1.7 μ m) using gradient elution with a mobile phase of acetonitrile and 0.1% formic acid in water at a flow rate of 0.4 mL/min. The detection was performed on a triple quadrupole MS/MS by multiple reaction monitoring mode to monitor the precursor-to-product ion transitions of m/z 436.1 → 145.1 for rivaroxaban, m/z 460.0 → 443.1 for apixaban, m/z 548.2 → 366.1 for edoxaban, and m/z 285.2 → 193.1 for diazepam (IS) using a positive ESI interface. The method was validated over a concentration range of 1.0–200 ng/mL for rivaroxaban, 1.0–100 ng/mL for apixaban, and 1.0–500 ng/mL for edoxaban. Total time for each chromatograph was 3.5 min. Reddy *et al.* [72] developed and validated a HPLC-MS/MS technique for the determination of rivaroxaban in human plasma. The samples were extracted using solid-phase extraction (SPE) technique wherein rivaroxaban D4 has been used as the internal standard. The use of isocratic LC method has enabled to achieve 2.0 min along with their respective internal standard using a Phenomenex Gemini C₁₈, 50×4.6 mm, 5 μ m column. ESI source in positive mode was used for the detections of rivaroxaban and IS. Precursor to product ion transition of m/z 436.20 → 144.80 for rivaroxaban and m/z 440.20 → 144.70 for IS was used in multiple reaction monitoring mode. Derogis *et al.* [73] proposed that a HPLC-MS/MS method was validated to measure rivaroxaban plasmatic concentration. In this method used a simple sample preparation, protein precipitation, and a fast chromatographic run. It was developed a precise and accurate method, with a linear range from 2 to 500 ng/mL, and a lower limit of quantification of 4pg on column. The new method was compared to a reference method (anti-factor Xa activity) and both presented a good correlation (r=0.98, p<0.001). In addition authors are validated hemolytic, icteric or lipemic plasma samples for rivaroxaban measurement by HPLC-MS/MS without interferences. The chromogenic and HPLC-MS/MS methods were highly correlated and should be used as clinical tools for drug monitoring. Puram *et al.* [74] evaluated the levels in plasma of pyridostigmine impurity B (metabolite of pyridostigmine) in humans after administration of pyridostigmine formulations. Plasma concentrations of pyridostigmine impurity B were estimated using by LC coupled with ESI triple quad mass spectrometer technique, lamivudine is used as an internal standard. Multiple reactions at 109.9/95.2 (pyridostigmine impurity B) and 230.2/112.2 (lamivudine) were monitored. Chromatography was optimized using acetonitrile: buffer (10 millimolar ammonium acetate) (85:15) on an Inertsil C18, 150 mm×4.6 mm, 5 μ analytical column. The Linear Calibration curve was ranges between 1.434 ng/ml and 39.637 ng/ml. Sample extraction was conducted using SPE extraction method using mixed mode cation exchange cartridges. The method was developed on API 4000. The method was developed, tested for system suitability, carryover, selectivity, matrix effect, intra-inter precision and accuracy, recovery, linearity, and various stabilities in aqueous as well as plasma as a matrix. The method was validated for all the above validation parameters mentioned as per European medical agency guideline on method validation. Yadav and Dubey [75] successfully developed a sensitive and accurate LC method for the quantification of rivaroxaban in human plasma. Protein precipitation was used for sample preparation. Quantification of analyte was achieved on phenomenon luna C₈ (5 μ m×25 cm×4.6 mm i.d.) column using methanol: water:dimethyl sulfoxide (50:45:5, v/v/v) as mobile phase at a flow rate of 1 ml/min. Detection was achieved at 252 nm over the linearity concentration range of 5–40 μ g/ml. Retention time was found 6.2 min. The Limit of detection and limit of quantification were found to be 1.5 μ g/ml and 4.5 μ g/ml, respectively. The developed method was validated as per the US FDA guidelines.

CONCLUSION

Most of the techniques such as spectrophotometric and chromatography RP-HPLC and LC coupled with MS/MS methods have been applied when dabigatran, rivaroxaban, and prasugrel are in pure form and its formulations. As far as, we know there is not a single research paper which refers to the quantification of dabigatran, rivaroxaban, and prasugrel by applicability quality by design. Hence, there is still scope for the development of new selective and specific analytical techniques for quantization of these molecules.

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