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Development and Validation of Simultaneous Estimation of Dabigatran Etexilate and Aspirin Using Vierodt's Uv Spectrophotometric and Rp-Hplc Techniques

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ABSTRACT

The present study describes the development and validation of novel, simple, precise, and reliable RP-HPLC and UV spectrophotometric methods for the simultaneous estimation of Dabigatran etexilate and Aspirin in combined form. The analytical procedures were developed and validated in accordance with ICH Q2(R2) guideline. For the UV spectrophotometric method, simultaneous equation analysis was performed using methanol as the diluent. The absorbance measurements were recorded at 225 nm (λmax of Dabigatran etexilate) and 276 nm (λmax of Aspirin). Chromatographic separation was achieved using an isocratic RP-HPLC method on a Kromstar C_{18} column (250 × 4.6 mm, 5 µm particle size). The mobile phase consisted of methanol and 0.05 M potassium dihydrogen phosphate buffer in the ratio of 70:30 (% v/v), delivered at a flow rate of 1.0 mL/min, with detection carried out at 230 nm. Both methods exhibited good linearity over the concentration range of 11-55 µg/mL for Dabigatran etexilate and 15-75 µg/mL for Aspirin. The developed methods were validated for specificity, linearity, precision, accuracy, sensitivity (LOD and LOQ), robustness, and assay and found to be reliable for routine quantitative analysis.

1. INTRODUCTION:

Dabigatran etexilate is an oral anticoagulant belonging to the class of direct thrombin inhibitors. It is indicated for the treatment and prevention of thromboembolic disorders, including pulmonary embolism, deep vein thrombosis, and the prevention of stroke and systemic embolism, particularly in patients with risk factors such as hypertension and hyperlipidemia[1]. Aspirin (acetylsalicylic acid) is classified as a non-steroidal anti-inflammatory drug (NSAID). It exerts its pharmacological effect by irreversibly inhibiting cyclooxygenase (COX-1 and COX-2) enzymes, thereby suppressing the synthesis of prostaglandins and thromboxane A_2. This action results in analgesic, antipyretic, anti-inflammatory, and antiplatelet effects[2].

The combination of Dabigatran etexilate with Aspirin was studied under clinical trial[3] phase and was proved that the combination is effective significantly in hemorrhagic pleural effusion in a patient with tuberculous pleuritis. This combination has been evaluated in clinical settings due to its complementary antithrombotic mechanisms[4]. Dabigatran etexilate directly inhibits thrombin (factor IIa), thereby preventing fibrin formation and thrombin-mediated platelet activation, whereas aspirin irreversibly inhibits cyclooxygenase-1 (COX-1), suppressing thromboxane A_2 mediated platelet aggregation. This dual pathway inhibition targets both primary and secondary haemostasis, offering broader protection against arterial and venous thromboembolic events[3-4]. In selected high-

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risk patients, such as those with atrial fibrillation and concomitant coronary artery disease, short-term combination therapy may reduce the risk of stroke, recurrent ischemic events, and stent thrombosis. However, despite its potential synergistic benefits, the increased risk of bleeding necessitates careful patient selection, dose optimization, and limitation of therapy duration[4-6].

A comprehensive literature survey revealed several reported analytical methods for the estimation of Dabigatran etexilate either alone or in combination with other drugs, including RP-HPLC methods[7-9], UV spectrophotometric methods[10-12], stability-indicating RP-HPLC methods[13-15], UPLC–MS/MS in human plasma[16], and UPLC/HPLC with fluorescence detection[17]. Similarly, numerous analytical approaches have been described for the quantitative determination of Aspirin, such as reversed-phase high-performance liquid chromatography[18], HPLC methods in human plasma[19-21], liquid chromatography–tandem mass spectrometry[22], and LC–MS/MS methods[23]. Despite the availability of these sophisticated and well-established analytical techniques, all reported methods focus on the individual estimation of these drugs or their determination in biological matrices. To the best of our knowledge, no validated spectrophotometric and chromatographic method has been reported for the simultaneous quantification of Dabigatran etexilate and Aspirin sodium in a synthetic mixture. The absence of a unified, cost-effective, and time-efficient analytical approach for their concurrent estimation highlights a significant analytical gap in the literature.

Therefore, the present study was undertaken to develop and validate novel, accurate, precise, and robust RP-HPLC and simultaneous equation UV spectrophotometric methods for the concurrent estimation of both drugs, in accordance with ICH Q2 (R2)[24] guideline, thereby providing a reliable analytical tool suitable for routine quality control analysis.

2. EXPERIMENTAL MATERIALS AND INSTRUMENTATION

2.1 Chemicals and reagents

Dabigatran etexilate was obtained as a gift sample from Cadila Pharmaceuticals Ltd., Dholka, Gujarat, India. Aspirin was procured from Intas Pharmaceuticals Ltd., Ahmedabad, Gujarat, India. HPLC-grade methanol was purchased from Finar Chemicals Pvt. Ltd., Ahmedabad, Gujarat, India. All other chemicals and reagents used in the study were of analytical reagent (AR) grade or HPLC grade and were used without further purification.

2.2 Instrumentation

UV spectroscopic analysis was performed using a Shimadzu UV-1900 UV–Visible spectrophotometer (Shimadzu Corporation, Kyoto, Japan) equipped with UV Probe 2.7 software, a spectral bandwidth of 1 nm, and 1.0 cm matched quartz cuvettes over the wavelength range of 200–400 nm.

Chromatographic analysis was carried out using a Systronics RP-HPLC system (Model SYS-LC-138, Systronics, India) coupled with a UV detector. The pH of the buffer solutions was measured using a Chemi Line pH meter (Chemi Line Instruments, India). An analytical balance (Scale-Tec, India) was used for accurate weighing of samples. The mobile phase was degassed by sonication using a Digital Pro+ sonicator (Model PS-10A, Broleo, India) prior to use.

2.3 Preparation of Solutions

2.3.1 Preparation of Stock Solution

Precisely weighed quantities of 10 mg each of Dabigatran etexilate and Aspirin were quantitatively transferred into a 100 ml volumetric flask and subsequently diluted to volume with methanol to achieve a final concentration of 100 µg/mL. The solutions were sonicated for 5 mins to ensure complete dissolution.

2.3.2 Preparation of calibration curve

The calibration standards in the concentration range of 11-55 µg/mL for Dabigatran etexilate and 15-75 µg/mL for Aspirin, appropriate aliquots of the respective stock solutions were transferred into a series of 10 mL volumetric flasks. For Dabigatran etexilate, aliquots of 1.1, 2.2, 3.3, 4.4, and 5.5 mL were diluted to volume with methanol to yield final concentrations of 11, 22, 33, 44, and 55 µg/mL, respectively. Similarly, for Aspirin, aliquots of 1.5, 3.0, 4.5, 6.0, and 7.5 mL were diluted to volume with methanol to obtain concentrations of 15, 30, 45, 60, and 75 µg/mL, respectively.

The prepared solutions were analyzed under optimized spectrophotometric conditions using a 1 cm matched quartz cuvette. For chromatographic analysis, 20 µL of each working standard solution was injected into the RP-HPLC system under optimized chromatographic conditions.

3. METHODOLOGY

3.1 Method I: UV SEPCTROPHOTOMETRIC METHOD DEVELOPMENT

Pipetted out 2.2 ml solution from stock solution of Dabigatran etexilate (100 µg/ml) and 3.0 ml Aspirin (100 µg/ml) into different 10 ml volumetric flask and diluted upto mark with methanol to get the 22 µg/ml of Dabigatran etexilate and 30 µg/ml Aspirin. Every solution was scanned between 200 to 400 nm.

3.1.1 Simultaneous equation as Vierordt's method

Solutions of Dabigatran etexilate (22 µg/ml) and Aspirin (30 µg/ml) prepared in methanol were subjected to a spectral scan from 200 to 400 nm at a medium speed, utilizing pure methanol as the reagent blank. For the analytical determination, the absorption maxima (λmax) were established at 225 nm for Dabigatran etexilate and 276 nm for Aspirin. This procedure applies the Simultaneous Equation technique based on Vierodt's principle, where the precise concentration of each drug within the sample is calculated according to the following mathematical expressions.

Standard Stock solutions of Dabigatran etexilate and Aspirin in the concentration range 11-55 µg/mL and 15-75 µg/ml were made in the methanol and absorbance of these solutions was measured at 225 nm and 276 nm. Calibration curves were plotted to confirm the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs. Two simultaneous equations as below were formed using these absorptivity values A (1%, 1 cm).

$$\text{At } \lambda 1 \; A1 = ax1bCx + ay1bCy \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$\text{At } \lambda 2 A2 = ax2bCx + ay2\, bCy \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

For measurements in 1 cm cells b=1,

Rearrange eq. (2),

$$Cy = A2 - ax2Cx/\, ay2$$

Substituting for Cy in eq (1) and rearranging

$$Cx = A2ay1 - A1\, ay2/ax2\, ay1 - ax1\, ay2 \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$Cy = A1ax2 - A2\, ax1/ax2\, ay1 - ax1\, ay2 \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where C_x and C_y are the concentration of Dabigatran etexilate and Aspirin, respectively, A_1 and A_2 are absorbance at 225 nm and 276 nm, respectively, ax_1 and ax_2 are absorptivity of Dabigatran etexilate at 225 nm and 276 nm, respectively; ay_1 and ay_2 are absorptivity of Aspirin at 276 nm and 225 nm, respectively. By solving the two simultaneous equations, the concentrations of Dabigatran etexilate and Aspirin in sample solutions were obtained.

3.2 Method II: Reverse Phase High Performance Liquid Chromatography Method

Chromatographic analysis was performed via isocratic elution, wherein various mobile phase configurations including Acetonitrile: Water, Methanol: Water, and Acetonitrile: buffer were evaluated in varying ratios. Optimal resolution of both analyte peaks was achieved using a mixture of Methanol and 0.05 M Potassium dihydrogen phosphate Buffer (70:30 %v/v) at a consistent flow rate of 1 mL/min. All solvents underwent filtration through a 0.45 µm membrane and were degassed via sonication for 30 minutes before use. Separation was executed on a Kromstar C_{18} (250 mm × 4.6 mm, 5 µm) stationary phase, with the eluent monitored using a UV Detector and chromatograms specifically extracted at 230 nm. Calibration curves were subsequently established by plotting the measured peak areas against their respective concentrations to derive the corresponding linear regression equations.

3.3 METHOD VALIDATION

The analytical methodologies employed in this research were rigorously validated in accordance with the regulatory standards established by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) under the ICH Q2 (R2)[24] guidelines for analytical procedure validation.

3.3.1 Specificity

Specificity denotes the capacity of an analytical procedure to accurately and distinctly quantify the target analyte despite the potential interference of co-existing substances. Within a complex sample, these extraneous components commonly encompass synthesis impurities, degradation products, or various matrix constituents that could otherwise confound the measurement.

3.3.2. Linearity and Range (n=6)

The linearity of the analytical procedure was evaluated through the preparation of five distinct concentrations of standard solutions. Dabigatran etexilate and Aspirin demonstrated linear responses within the concentration ranges of 11-55 µg/mL and 15-75 µg/mL, respectively. The proportionality of both analytes was statistically assessed by calculating the slope, y-intercept, and correlation coefficient (R^2) from the resulting calibration curves.

3.3.3. Precision

The precision of both analytical methodologies was evaluated across three distinct parameters: repeatability, intraday (intermediate) precision, and interday (reproducibility) precision. To assess intraday precision, standard solutions of Dabigatran etexilate (11, 22, 33 µg/mL) and Aspirin (15, 30, 45 µg/mL) were analyzed in triplicate at three separate time intervals within a single day. Interday precision was similarly established by evaluating the same concentration levels over three consecutive days. Furthermore, repeatability was rigorously determined through six replicate injections of a single concentration level 22 µg/mL for Dabigatran etexilate and 30 µg/mL for Aspirin. All precision data were statistically quantified and reported as the percentage relative standard deviation (%RSD) to ensure compliance with ICH Q2 (R2) guideline.

3.3.4 Limit of Detection (LOD):

Limit of detection can be calculated using following equation as per ICH guidelines.

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$$LOD = 3.3 * \frac{\sigma}{S}$$

Where, σ = standard deviation of the calibration curve

S = slope of the calibration curve

3.3.5 Limit of Quantification (LOQ):

Limit of quantification can be calculated using following equation using the standard deviation of the Y-intercept (σ) and the mean slope (S) of the calibration curve according to ICH Q2 (R2) guideline.

$$LOQ = 10 * \frac{\sigma}{S}$$

Where, σ = standard deviation of the calibration curve

S = slope of the calibration curve

3.3.6 Accuracy (Recovery study) (n=3)

The accuracy of an analytical procedure denotes the proximity of the experimental result to the accepted reference value or conventional true value. To confirm the accuracy of the proposed method, recovery studies were conducted in accordance with ICH Q2(R2) Guidelines at three distinct concentration levels: 50%, 100%, and 150%. These evaluations targeted Dabigatran etexilate (22 µg/ml) and Aspirin (30 µg/ml) using the standard addition technique, with each level analyzed in triplicate. The methodology's accuracy was subsequently established by calculating the percentage recovery of both analytes across these fortified concentrations.

3.3.7 Assay as analysis of Synthetic Mixture

A synthetic mixture containing Dabigatran etexilate and Aspirin in the ratio of 11:15 was prepared. Accurately weighed quantities of Dabigatran etexilate (22 mg) and Aspirin (30 mg) were blended with commonly used excipients, namely microcrystalline cellulose (15 mg), lactose (11 mg), magnesium stearate (10 mg), talc (6 mg), and croscarmellose sodium (6 mg), using a mortar and pestle to obtain a homogeneous mixture. An accurately weighed portion of the prepared blend equivalent to 22 mg of Dabigatran etexilate and 30 mg of Aspirin was transferred into a 100 mL volumetric flask. Approximately 70 mL of methanol was added, and the mixture was sonicated to ensure complete dissolution of the drugs. The volume was then made up to the mark with methanol and mixed thoroughly. The resulting solution was filtered through Whatman filter paper to remove insoluble excipients. The obtained stock solution contained 220 µg/mL of Dabigatran etexilate and 300 µg/mL of Aspirin. For sample analysis, 1.0 mL of this stock solution was accurately transferred into a 10 mL volumetric flask and diluted to volume with methanol to yield final concentrations of 22 µg/mL of Dabigatran etexilate and 30 µg/mL of Aspirin. The prepared sample solution was analyzed using the optimized RP-HPLC and UV spectrophotometric methods, and the percentage assay of both drugs was calculated.

3.3.8 Robustness

Robustness of the developed RP-HPLC and UV spectrophotometric methods was evaluated by deliberately introducing small and systematic variations in analytical conditions and assessing their effect on the assay results. For the RP-HPLC method, robustness was examined by varying the flow rate (±0.1 mL/min from 1.0 mL/min), mobile phase composition (±2% variation in organic phase), and detection wavelength (±2 nm from 230 nm). The effects of these changes on retention time, peak area, tailing factor, and resolution were studied. For the UV spectrophotometric method, robustness was assessed by varying the detection wavelength (±2 nm from 225 nm for Dabigatran etexilate and 276 nm for Aspirin) and evaluating the effect of slight variations in solvent composition.

3.3.9 System Suitability Tests

A system suitability test is an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. The test includes the Resolution, Column efficiency, Tailing factor and Theoretical plates (table 1).

4. RESULTS AND DISCUSSION

4.1 Selection of wavelength

For the simultaneous equation method, standard solutions of Dabigatran etexilate (22 µg/mL) and Aspirin (30 µg/mL) in methanol were subjected to spectral scanning between 200 and 400 nm at medium speed, with methanol employed as the blank solution. For the analytical determination, the absorption maxima (λmax) were established at 225 nm for Dabigatran etexilate and 276 nm for Aspirin (Figure 1). This procedure applies the Simultaneous Equation technique based on Vierodt's principle, where the precise concentration of each drug within the sample is calculated. For RP-HPLC method, coupled with UV detection, is fundamentally dependent upon the strategic selection of an optimal detection wavelength. Both analytes exhibited significant molar absorptivity at 230 nm, leading to its selection for the simultaneous quantification of Dabigatran etexilate and Aspirin within the synthetic mixture. The spectral rationale for this choice of detection wavelength is showed in Figure 1.

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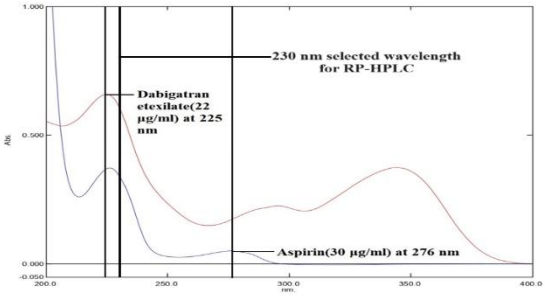

Figure 1: Overlain UV Spectra of Dabigatran etexilate (22 µg/mL) and Aspirin (30 µg/mL) in methanol

*4.2 Simultaneous equation (*Vierordt's*) method*

For multi-component UV analysis, Vierordt's method is named after the German scientist Karl Vierordt. UV Spectra of Dabigatran etexilate (11-55 µg/mL) and Aspirin (15-75 µg/mL) over the linearity and range had been showed in Figure 2 and 3, respectively.

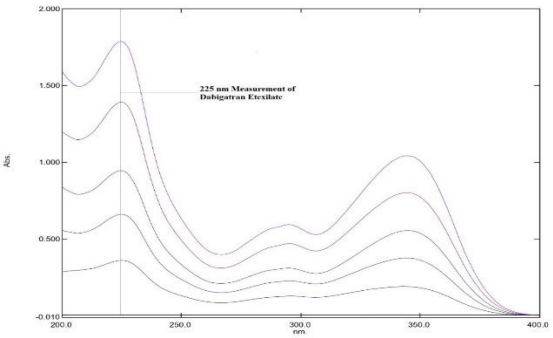

Figure 2: Overlain UV Spectra of Dabigatran etexilate (11-55 µg/ml) at 225 nm

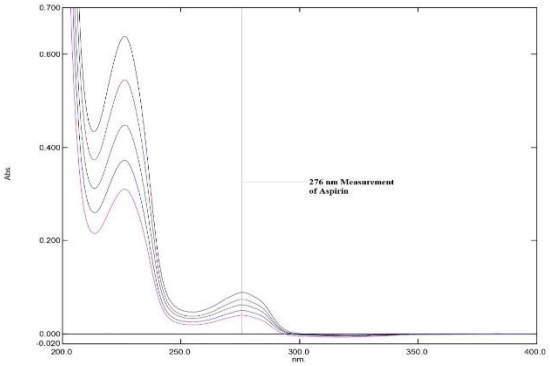

Figure 3: Overlain UV Spectra of Aspirin (15-75 µg/ml) at 276 nm

4.3 RP-HPLC Method Development

An RP-HPLC method coupled with UV detection was developed for the concurrent quantification of Dabigatran etexilate and Aspirin, with the primary objective of achieving optimal peak symmetry and high theoretical plate counts within an efficient analytical runtime. Chromatographic parameters were refined through the systematic evaluation of various stationary and mobile phase compositions. Among the reversed-phase C_8 and C_{18} columns assessed, the Kromstar C_{18} (250 × 4.6 mm, 5 µm) demonstrated superior performance, yielding highly symmetric peaks and the most favorable retention times. The optimal mobile phase was identified as a mixture of Methanol and 0.05 M Potassium dihydrogen phosphate Buffer (70:30 %v/v) at 230 nm. Although alternative ratios of this buffer and solvent were investigated, they resulted in undesirable peak tailing and excessive retention for both analytes.

4.4 VALIDATION OF THE PROPOSED METHODS

4.4.1 Specificity

Specificity is defined as the ability of an analytical method to unequivocally assess the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. The specificity of the developed RP-HPLC method was evaluated by comparing chromatograms of the mobile phase (blank), placebo (excipients), and the test preparation solution. The chromatogram of the blank showed no peaks at the retention times corresponding to Dabigatran etexilate and Aspirin. Similarly, no interfering peaks from excipients were observed at the respective retention times of the analytes in the sample chromatogram. These results demonstrate that the developed method is specific and free from interference due to mobile phase components or formulation excipients, thereby confirming its suitability for the simultaneous estimation of Dabigatran etexilate and Aspirin. Retention time was found to be 4 min and 6.3 min for Dabigatran etexilate and Aspirin, respectively showed in table 1.

Table 1: System suitability parameters for Dabigatran etexilate and Aspirin

Sr. No.	System suitability parameters	Dabigatran etexilate	Aspirin
1.	Retention time	4.0 min	6.3 min
2.	Theoretical Plates	3011.33	4835.55
3.	Tailing Factors	1.10	1.30
4.	Resolution	4.2	

4.4.2 Linearity and range

UV Spectra of Dabigatran etexilate (11-55 µg/ml) and Aspirin (15-75 µg/ml) over the linearity and range had been showed in Figure 2 and 3, respectively. For UV, Dabigatran etexilate exhibited a linear response in the concentration range of 11–55 µg/mL at 225 nm and 276 nm. The correlation coefficients (r^2) were found to be 0.9984 and 0.9989 at 225 nm and 276 nm, respectively, indicating excellent linearity. The mean absorbance values (n = 6) showed low standard deviation with %RSD values below 1.5%, demonstrating good precision and repeatability. Aspirin showed linearity over the concentration range of 15-75 µg/mL at 276 nm and 225 nm, with correlation coefficients (r^2) of 0.9990 and 0.9992, respectively.

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The RP-HPLC chromatogram of Dabigatran etexilate (11-55 µg/mL) and Aspirin (15-7 5 µg/mL). The Peak Area was found. Calibration graphs were plotted between concentrations and peak areas were observed. The regression equation of calibration curve was generated and Correlation Coefficient for Dabigatran etexilate 0.9996 and for Aspirin 0.9998, respectively. The %RSD values were less than 2.0%, confirming acceptable precision and reproducibility of the developed method. The linearity data are summarized in Table 2.

Table 2: Linearity and sensitivity data of Dabigatran etexilate and Aspirin

Parameters	UV Spectrophotometry				HPLC	
	Dabigatran etexilate		Aspirin		Dabigatran etexilate	Aspirin
Wavelength (nm)	225 nm	276 nm	276 nm	225 nm	230 nm	
Beer's Law Limit (µg/mL)	11-55	11-55	15-75	15-75	11-55	15-75
Correlation Coefficient (r^2)	0.9984	0.9989	0.999	0.9992	0.9996	0.9998
LOD	0.14	0.38	2.93	0.76	0.38	0.02
LOQ	0.43	1.17	8.88	2.31	1.26	0.07

4.4.2.1 Calculation for Simultaneous Equation Method for Dabigatran etexilate and Aspirin in Synthetic Mixture.

Dabigatran etexilate (22 µg/ml) and Aspirin (30 µg/ml) in methanol, both the solutions were scanned over range of 200-400nm against methanol as blank, using medium scan speed. The sampling wavelength for analysis includes 225 nm for Dabigatran etexilate and 276 nm for Aspirin. The method employs Simultaneous Equation as per Vierodt's method and the concentrations of drugs in sample solution were determined by using the following formula:

Dabigatran etexilate,

$$C_x = \frac{A2 \times ay1 - A1 \times ay2}{ax2 \times ay1 - ax1 \times ay2}$$

Where ax_1 and ax_2 represented the absorptivity of Dabigatran etexilate at 225 nm and 276 nm, respectively; ay_1 and ay_2 denoted the absorptivity of Aspirin at 276 nm and 225 nm, respectively; and A_1 and A_2 corresponded to the absorbance of the sample measured at 225 nm and 276 nm, respectively.

C (Dabigatran etexilate) = 21.98 µg/mL; The concentration of Dabigatran etexilate (Cx), calculated using Vierordt's simultaneous equation method, was found to be 21.98 µg/mL.

For Aspirin,

$$C_y = \frac{A1 \times ax2 - A2 \times ax1}{ax2 \times ay1 - ax1 \times ay2}$$

where a_{x1} and a_{x2} are the absorptivity values of Dabigatran etexilate at 225 nm and 276 nm, respectively; a_{y1} and a_{y2} represent the absorptivity of Aspirin at 276 nm and 225 nm, respectively; and A_1 and A_2 are the absorbance values of the sample measured at 276 nm and 225 nm, respectively.

C (Aspirin) = 29.18 µg/mL; The concentration of Aspirin (Cy), calculated using Vierordt's simultaneous equation method, was found to be 29.18 µg/mL.

4.4.3 Precision

Methodological precision was evaluated through intraday, inter-day, and repeatability assessments using triplicate analyses of Dabigatran etexilate (11, 22 and 33 µg/ml) and Aspirin (15, 30 and 45 µg/ml) across three consecutive days and within a single diurnal period. Absorbance values were recorded for these concentrations to establish intermediate precision, while repeatability was specifically determined using concentrations of 22 µg/ml for Dabigatran etexilate and 30 µg/ml for Aspirin. The outcomes, expressed as Relative Standard Deviation (% RSD) for each precision parameters were less than two.

4.4.4 LOD and LOQ

The limits of detection (LOD) and quantification (LOQ) are calculated using the standard deviation responses and slopes obtained from the calibration curves of each drug at their specific wavelengths. The results of LOD and LOQ were displayed in Table 2.

4.4.5 Accuracy

To evaluate the accuracy of the proposed methodology, recovery studies were performed using the standard addition technique, in which pre-analyzed samples were spiked with known concentrations of pure Dabigatran etexilate and Aspirin. These assessments were executed at three levels 50%, 100%, and 150% and conducted in triplicate to ensure statistical reliability. The accuracy was expressed as the percentage recovery of the added standards. For the UV spectrophotometric approach, the percentage recovery was found to be within range of 99.64%-99.93% for Dabigatran etexilate and 99.33%-99.73% for Aspirin. For RP-HPLC method, the percentage recovery was found to be within the

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range of 99.90%-100.01% for Dabigatran etexilate and 99.70%-100.04% for Aspirin, with detailed results provided in Table 3.

Table 3: Recovery study data for UV and RP-HPLC Method

Vierordt's Method						
Name of Drug	**% Level of recovery**	**Test Amount (µg/mL)**	**Amount of drug taken (µg/mL)**	**Total Std Amt (µg/mL)**	**Total amount Recovered (µg/mL)**	**% Mean Recovery ± SD(n=3)**
Dabigatran etexilate	50	22	11	33	32.97	99.64 ±0.330
	100	22	22	44	43.98	99.83±0.076
	150	22	33	55	55.01	99.93±0.023
Aspirin	50	30	15	45	45.02	99.33±0.330
	100	30	30	60	59.99	99.58±0.144
	150	30	45	75	74.98	99.73±0.115
RP-HPLC Method						
Dabigatran etexilate	50	22	11	33	32.97	99.90±0.0557
	100	22	22	44	43.98	99.95±0.01
	150	22	33	55	55.01	100.01±0.011
Aspirin	50	30	15	45	45.02	100.04±0.0057
	100	30	30	60	59.99	99.98±0.01
	150	30	45	75	74.98	99.70±0.015

4.4.6 Assay as Analysis of Synthetic mixture

From assay, the concentration of Dabigatran etexilate 22 µg/mL and Aspirin 30 µg/mL were run into UV and RP-HPLC. The Percentage assay of Dabigatran etexilate and Aspirin were found to be 99.86% and 99.93% respectively in UV. For RP-HPLC the Percentage assay of Dabigatran etexilate and Aspirin were found to be 99.90% and 99.96%, respectively. Its results showed in Table 4.

Table 4: Assay results for UV and RP-HPLC Method

Vierordt's Method				
Name of Drug	**Amount in synthetic mixture (µg/mL)**	**Mean Amount found (µg/mL)**	**% Assay ± SD (n=3)**	**%RSD**
Dabigatran etexilate	22	21.97	99.86 ± 0.251	0.253
Aspirin	30	29.98	99.93 ± 0.288	0.291
RP-HPLC Method				
Dabigatran etexilate	22	21.98	99.90 ± 0.07	0.07
Aspirin	30	29.99	99.96 ± 0.04	0.04

4.4.7 Robustness

Chromatographic analysis was used to analyses the effects of changes in analysts, and the results showed that there was no statistically significant difference in the % RSD of technique II. Additionally, small changes were performed to assess the robustness of the created HPLC procedures. The approaches' robustness was demonstrated by the% RSD, which remained constant despite minor variations in flow rate, run time, and detection. It was determined that the created approaches were essential. The results indicated that minor deliberate variations in method parameters did not produce significant changes in analytical responses. The percentage relative standard deviation (%RSD) values were found to be within acceptable limits (<2%), demonstrating that the developed methods are robust and reliable for routine analysis.

5. CONCLUSION:

The current investigation focused on the development and validation of streamlined, cost-effective, and precise analytical protocols for the concomitant quantification of Dabigatran etexilate and Aspirin in a synthetic matrix. While previous literature documents various techniques for these analytes in isolation, a literature gap was identified regarding their simultaneous determination. Consequently, UV-Spectrophotometric and RP-HPLC methodologies were established and validated in strict accordance with ICH Q2 (R2) regulatory standards. For the UV-Spectrophotometric approach, the Vierordt's (simultaneous equation) method was employed, utilizing analytical wavelengths of 225 nm and 276 nm for Dabigatran etexilate and Aspirin, respectively. This method exhibited robust linearity over concentration intervals of 11-55 µg/ml and 15-75 µg/ml, respectively, yielding correlation coefficients nearer to 0.999. Comprehensive validation encompassing accuracy, precision, repeatability, and sensitivity (LOD/LOQ) yielded results within established acceptance criteria. Furthermore, recovery experiments and assay data substantiated the method's reliability for estimating both components within the synthetic mixture. Additionally, a highly sensitive RP-HPLC method was optimized using a C_{18} stationary phase and a mobile phase comprised of Methanol: 0.05 M Potassium dihydrogen phosphate buffer (70:30 % v/v). The system operated at a flow rate of 1 ml/min with UV detection at 230 nm, resulting in well-

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resolved peaks and favorable system suitability metrics. The chromatographic technique demonstrated superior linearity, precision, and robustness. The obtained assay percentages confirmed that this method is highly suitable for standardized quantitative assessments. In conclusion, both newly developed analytical platforms proved to be efficient, accurate, and reproducible. These validated methods are highly recommended for routine quality control and the simultaneous monitoring of Dabigatran etexilate and Aspirin in both synthetic mixtures and commercial pharmaceutical formulations.

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CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

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