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Research Article

HPLC Method Development and Validation for the Determination of Apixaban and Clopidogrel in Novel Fixed-Dose Combination Tablets

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A simple, fast, and accurate high-performance liquid chromatographic (HPLC) method is developed, optimized, and validated for a fixed-dose combination of apixaban (APX) and clopidogrel (CLOP) tablets according to ICH guidelines. Chromatographic separation of the drugs was performed on a BDS Hypersil C_{18} (4.6 ∗ 150 mm, 5 μm), with acetonitrile (ACN) and trifluoroacetic acid (TFA) in the ratio 48 : 52 (v/v) as a mobile phase, at a flow rate of 0.9 ml/min., injection volume of 5 μL, and column temperature 45°C. The proposed method was linear over the level 25–200% for a concentration of APX 5 μg/ml and CLOP 75 μg/ml ($R^2 > 0.999$). The detection limit for APX and CLOP was found to be 0.3465 and 3.8496 μg/ml, whereas the quantification limit was 1.0499 and 11.6656 μg/ml, respectively. The recovery was more than 99% using the standard addition method. The developed method was found to be specific, accurate, precise, and robust against changes in column temperature (±5°C) and mobile phase composition (±5% ACN); hence, it can be used for the determination of APX and CLOP in the fixed-dose combination tablets.

1. Introduction

Apixaban (Eliquis®) is an oral anticoagulant agent manufactured by Bristol-Myer Squibb S.R.L, Pfizer Limited. It is a potent, reversible, direct, and highly selective active site inhibitor of factor Xa [1, 2]. It prevents thrombin generation and thrombus development by inhibiting free and clot-bound factor Xa and prothrombinase activity. Apixaban's (APX's) chemical name is a 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl) phenyl]-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c] pyridine-3-carboxamide [1, 2]. It is a nonhygroscopic crystalline powder, a nonionizable compound, stable, and not sensitive to heat, light, or moisture [1]. APX's aqueous solubility across the physiological pH range is ~0.04 mg/ml, and it is classified according to the biopharmaceutical classification system (BCS) as a class III drug; a highly soluble low permeable substance. The structure is shown in Figure 1(a) [3].

Clopidogrel (Plavix®) is an antiplatelet prodrug manufactured by Sanofi. It works as a platelet aggregation inhibitor by inhibiting adenosine diphosphate binding to its receptor, and the subsequent ADP-mediated activation of the glycoprotein GPIIb/IIIa complex. It is synthesized as clopidogrel hydrogen sulfate salt. Chemically, it is methyl (+)-(S)-α-(2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate sulfate (1 : 1). The chemical structure of CLOP hydrogen sulfate salt is shown in Figure 1(b) [4]. Clopidogrel (CLOP) is a white to off-white powder, its solubility is affected by pH, and it is freely soluble in water at pH 1 [5, 6].

Combined use of anticoagulant and antiplatelet medications is common among comorbid cardiovascular patients, including atrial fibrillation (AFib) and recent acute coronary syndrome (ACS) or percutaneous coronary intervention (PCI). They are used as prophylaxis and treatment of thromboembolic events [7–9]. In addition, the

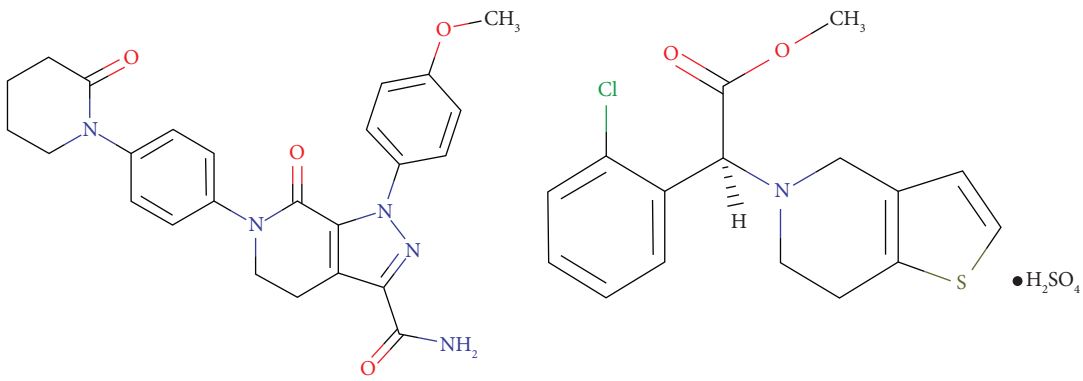


Figure 1: Chemical structure of (a) apixaban and (b) clopidogrel hydrogen sulfate.

formulation of fixed-dose combination (FDC) may enhance their pharmacological effect [10].

A FDC of APX and CLOP has been developed in the current study, with APX having an extend-release (ER) tablet and CLOP having an immediate-release (IR) tablet encapsulated within translucent gelatin shell capsules. While the two active ingredients are available in the market as two separate IR dosage forms, a literature search was conducted to find an analysis method for the combination. The search revealed that there is no official monograph of APX in any pharmacopoeia, and several analytical methods have been developed in labs to determine the amount of APX individually [11–14] or in combination with other drugs [15] using high-performance liquid chromatography (HPLC). Regarding CLOP, an official monograph was published for it in the USP and revealed that CLOP could be analyzed using a special column for HPLC analysis, and the absorbance is measured at 220 nm [16]. In addition, many studies have aimed to develop different methods of analysis of CLOP individually [17, 18] or in combination using different technologies such as HPLC [19–21], ultra-high performance liquid chromatography (UHPLC) [22], UV spectrophotometric method [23], and thin layer chromatography (TLC) [24]. When performing our research, none of these studies had developed an analytical method for simultaneously determining APX and CLOP in the same dosage form using any spectrophotometric method.

HPLC is an analytical method that is commonly used to separate, identify, and quantify the amount of each component in a mixture. It is identified as the most accurate analytical method for the quantitative and qualitative analysis of drug products [25, 26].

Developing an accurate analytical method is a crucial step during the development process of a new formula, and the study aimed to develop a simple, rapid, and accurate HPLC analytical method for quantification of the amount of APX and CLOP in a novel FDC dosage form. The method was validated according to the International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use guidelines by assessing its specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and robustness.

2. Materials and Methods

2.1. Materials and Reagents. A pharmaceutical grade APX and CLOP were received as gifts from Pharmacare PLC (Palestine). HPLC grade of acetonitrile (ACN), trifluoroacetic acid (TFA), and triethylamine (TEA) were purchased from Merck (Merck Serono Amman, Jordan). Tablet excipients including hydroxypropyl methylcellulose, 28–30% methoxyl, 7–12% hydroxypropyl, viscosity (2% aq. soln., 20°C) 7500–14000 MPa·s (HPMC), and hydroxypropyl cellulose (HPC) were purchased from 9 Alfa Aesar (Thermo Fisher Scientific, United Kingdom). Methocel, sodium laurilsulfate (SLS), lactose spray dried, colloidal silica, sodium stearyl fumarate (SSF), and magnesium stearate (Mg.St.) were donated from Pharmacare PLC (Palestine). Water was obtained by filtration using a cellulose nitrate filter (0.45) micron manufactured by Sartorius Stedim Biotech Company (Jordan).

2.2. Instruments. Analysis was carried out by Agilent HPLC 1200 series with AS thermostat (Santa Clara, USA), equipped with a pump model (G1312A), an autosampler (ALS) model (G1329A), and a UV/VIS detector. The used column was BDS Hypersil C_{18} (4.6 ∗ 150 mm), 5 μm, Thermo Scientific part #28105–154630. PerkinElmer® double beam UV/VIS spectrometer Lambda 25, Ohaus® electronic balance, Elma® sonicator, and vacuum filter pump were also used during the analysis.

2.3. Selection of Wavelength. 5 mg of APX and 98 mg of CLOP hydrogen sulfate salt equivalent to 75 mg CLOP were dissolved in 100 ml of ACN. From this solution, 5 ml was obtained and diluted in a 50 ml volumetric flask using distilled water (DW) to give the standard with 0.005 and 0.075 mg/ml of APX and CLOP, respectively. A blank was prepared by mixing ACN : DW, 5 : 50 (v/v). A scan for the

wavelength with a good absorbance was performed between 200 and 400 nm.

2.4. Chromatographic Conditions. The buffer was prepared by dissolving 0.5 ml of TFA in 1000 ml of DW and then the pH was adjusted to 2.2 by adding TEA. The final mobile phase was prepared by mixing 480 ml ACN with 520 ml buffer. Before use, the mobile phase was filtered using 0.45 μm cellulose nitrate filters. The injection volume was 5 μL, the mobile phase flow rate was 0.9 ml/min., and the column temperature was set at 45°C.

2.5. Preparation of Fixed-Dose Combination (APX and CLOP) Tablets. The dosage form was prepared as two small tablets encapsulated within a gelatin capsule. APX tablets were prepared as extended-release tablets with a dose of 5 mg. It was prepared by the wet granulation method. The formulation comprised of APX, HPMC, HPC, Methocel, SLS, and Mg.St.

For CLOP, it was prepared as immediate-release tablets with a dose equivalent to 75 mg of CLOP using the direct compression method. The formulation comprised CLOP, HPC (Klucel®), spray dried lactose, colloidal silica, and SSF.

2.6. Preparation of Stock and Standard Solutions. A stock solution with a concentration of 0.05 and 0.75 mg/ml of APX and CLOP was prepared by weighing 5 gm of APX and 98 mg of CLOP salt equivalent to 75 mg CLOP and dissolved in 100 ml HPLC grade ACN. Then, it was sonicated for 5 min. to obtain a standard solution of a mixture of APX and CLOP.

2.7. Preparation of Sample Solutions. Ten FDC (APX and CLOP) tablets were weighed, placed in the mortar, and finely powdered. The tablet powder equivalent to 5 mg APX and 75 mg CLOP was transferred into a 100 ml volumetric flask. About 40 ml of ACN was added to the flask and shaken vigorously. The volume was made up to 100 ml with ACN and sonicated for 10 min. Then, the contents were filtered through syringe filters of 0.45 μm (Clarify®). From this sample stock solution, 5 ml was transferred into a 50 ml volumetric flask. The volume was made up to the mark with DW. The prepared solution was injected into the HPLC to obtain the APX and CLOP content percentage in the tablets.

2.8. Preparation of Placebo Solution. The placebo solution for FDC tablets was prepared by mixing all the excipients (HPMC, HPC, Methocel, SLS, spray dried lactose, colloidal silica, SSF, and Mg.St. in ACN), then 5 ml were transferred into a 50 ml volumetric flask, and volume was completed by DW.

2.9. Method Validation. The proposed analytical method was validated concerning parameters such as specificity, linearity, range, accuracy, precision, LOD, LOQ, and robustness as described in ICH guidelines Q2 (R1) [27] and the FDA guidance [28, 29].

2.9.1. Specificity. Specificity is known as the ability of the method to measure the analyte accurately and without interference from other expected components and is considered one of the significant features of the HPLC method [27]. The specificity of the method was determined by injecting blank, placebo, standard, and sample solutions separately and recording the chromatograms using the proposed method.

2.9.2. Linearity, Range, and Sensitivity. From the stock solution, different aliquots of standard solution equivalent to 0.00125, 0.0025, 0.004, 0.005, 0.0075, and 0.01 mg/ml of APX and 0.01875, 0.0375, 0.06, 0.075, 0.1125, and 0.15 mg/ml of CLOP were transferred into a series of volumetric flasks, and the volume was completed with DW. Next, the solutions were injected in triplicate into the HPLC column. Then, calibration curves were plotted against the final concentrations and the regression equations were obtained.

To assess the sensitivity, LOD and LOQ of the method were calculated according to the following formula based on the calibration curve.

$$\begin{aligned} \mathrm{LOD} &= 3.3 * \frac{\sigma}{S}, \\ \mathrm{LOQ} &= 10 * \frac{\sigma}{S}, \end{aligned} \tag{1}$$

where σ = the response standard deviation and S = the slope of the calibration curve.

2.9.3. Accuracy. A recovery study evaluated the accuracy. It was evaluated by the standard addition method that a mixture of the used excipients solution was prepared with three known concentrations of APX and CLOP reference standards in triplicate. Nine samples were injected and the % recovery and % RSD were calculated for each replicate sample.

2.9.4. Precision. Precision was evaluated in terms of system and method repeatability. To assure system repeatability, 10 injections were performed on a freshly prepared stock solution under the proposed chromatographic condition on the same day to evaluate the system precision. Also, to assure method precision, intra- and interday studies were carried out. Intraday precision was studied by analyzing six replicates of prepared samples of tablets preparation within the same day. The interday precision was checked by analyzing the same concentration of prepared samples of tablets on three different days. Mean and % RSD were calculated for intra- and interday studies.

2.9.5. Robustness. Evaluation of robustness was achieved by making some small deliberate changes in the method parameters. It was carried out on three replicates of standard

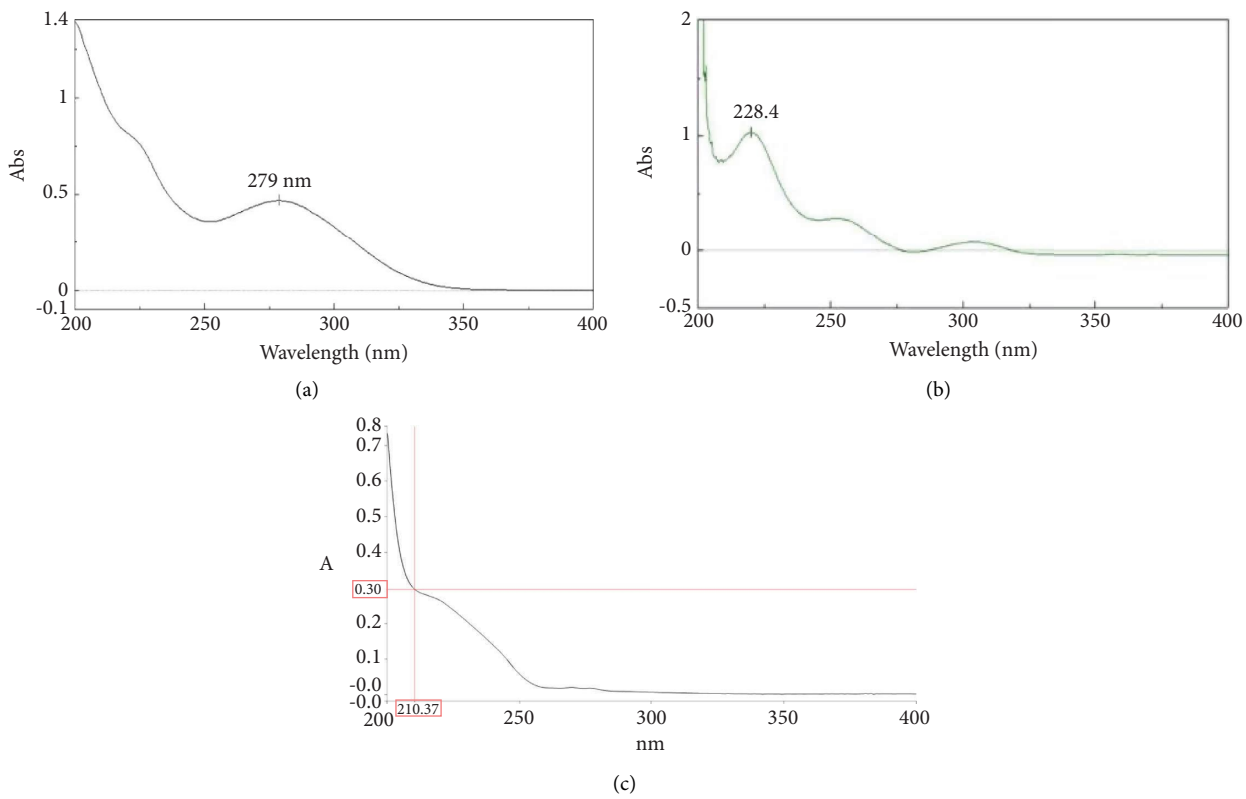


Figure 2: UV spectra of (a) apixaban, (b) clopidogrel hydrogen sulfate, and (c) apixaban and clopidogrel hydrogen sulfate standards in 40 ACN:60 DW.

drug solution. The effects of modifying the flow rate (±0.3 mL/min), column temperature (±5°C), mobile phase composition (±5% ACN), and wavelength (±5 nm) were studied. One parameter was changed per trial to estimate its effect on the retention time, peak area, and tailing factors.

2.9.6. Stability of Analytical Solution. The analytical solution stability was determined by analyzing a triplicate of standard and sample preparations in a refrigerator and at ambient room temperature upon preparation, and after 48 hrs, % RSD of the peak was calculated.

3. Results and Discussion

3.1. Selection of Wavelength. The absorption spectra of APX and CLOP showed a good absorbance detected at a wavelength around 279 nm for APX and 228.4 nm for CLOP, as shown in Figures 2(a) and 2(b), respectively. The maximum absorbance for the mixture was detected at 210 and 223 nm, as shown in Figure 2(c). After analyzing the mixture at different wavelengths, including 205, 210, 215, 220, and 223 nm using HPLC, the selected λ max was 210 nm for dual analysis of both APIs in the same run, as the mixture of drugs showed significant absorbance and fewer noises at the baseline of HPLC spectra.

3.2. Selection of HPLC Chromatographic Conditions. To develop the analytical method, different chromatographic conditions were examined to have two well-resolved sharp peaks, with acceptable resolution time and tailing factor within the accepted values. For this purpose, a series of trials were performed by verifying the column type, mobile phase type, ratio, pH, flow rate, column temperature, wavelength, and injection volume. Table 1 summarizes the results of method development.

The final chromatographic conditions were set using an acidic mobile phase containing ACN: 0.05% v/v TFA, 48 : 52 (v/v), and pH 2.2. The pKa of APX is 13.12 [30] and 4.6 for CLOP [31]; as the compounds' pKa values are higher than the mobile phase pH, compounds are expected to be in their nonionizable form and peaks with better resolution would be obtained [30]. The injection volume was 5 μL, the mobile phase flow rate was 0.9 ml/min., the column temperature was set to 45°C, the wavelength was λ 210 nm, and the run time was set at 10 min. The resulting peaks were eluted forming two sharp peaks, almost symmetric in shape with

Table 1: Chromatographic condition scanning trials.

Mobile phase % (v/v)	Flow rate (ml/min)	Injection volume (μL)	Wavelength (nm)	Column temp. (°C)	Mobile phase pH	Observation	Result
DW. : ACN, 60 : 40	1	20	205	Ambient	NM	No peaks	Rejected
DW. : ACN, 60 : 40	1	20	224	Ambient	NM	No peaks	Rejected
DW. : ACN, 40 : 60	1	20	224	Ambient	NM	1 peak	Rejected
DW. : ACN, 20 : 80	1	20	224	Ambient	NM	Unresolved peaks	Rejected
PB. : ACN, 60 : 40	1	20	224	Ambient	NM	2 peaks, TF > 2 for CLOP	Rejected
PB. : ACN, 50 : 50	1	20	224	Ambient	NM	2 peaks, TF > 2 for CLOP	Rejected
PB. : ACN, 30 : 70	1	20	224	Ambient	NM	Very short run time (LT 3 minutes.)	Rejected
PB. : ACN, 35 : 65	1	20	224	Ambient	NM	2 peaks, TF > 2 for CLOP	Rejected
PB. : ACN, 35 : 65	1	10	224	Ambient	NM	2 peaks, TF > 2 for CLOP	Rejected
PB. : ACN, 35 : 65	1	10	224	45	NM	2 peaks, TF > 2 for CLOP	Rejected
PB. : ACN, 35 : 65	1	5	224	45	NM	Poor Abs. for APX	Rejected
PB. : ACN, 35 : 65	1	5	214	45	NM	Poor Abs. for APX and CLOP	Rejected
PB. : ACN, 35 : 65	1	5	205	45	NM	Very poor Abs. for APX	Rejected
PB. : ACN, 35 : 65	1	5	214	45	NM	Poor Abs. for both	Rejected
PB. : ACN, 35 : 65	1	5	224	45	NM	Poor Abs. for both	Rejected
TFA : ACN, 35 : 65	1	5	224	45	2	Short run time (LT 3 min)	Rejected
TFA : ACN, 55 : 45	1	5	224	45	2	Poor Abs. for APX	Rejected
TFA : ACN, 52 : 48	0.9	5	224	45	2.2	Noises on the baseline HPLC spectra	Rejected
TFA : ACN, 52 : 48	0.9	5	210	45	2.2	Proposed	Accepted

DW.: distilled water, ACN: acetonitrile, PB.: phosphate buffer, TFA.: trifluoroacetic acid buffer, TF.: tailing factor, Abs.: absorption, NM.: not measured.

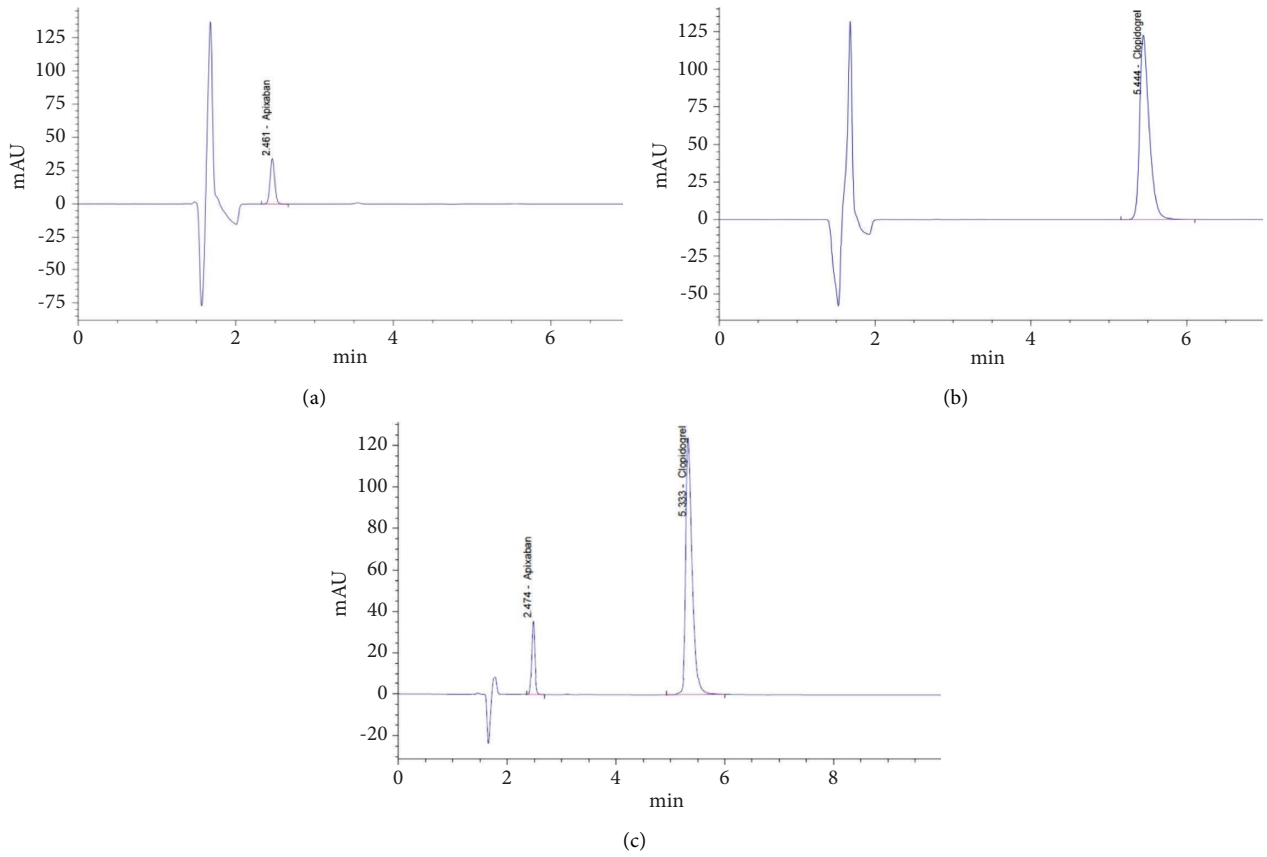


Figure 3: Chromatogram of (a) apixaban, (b) clopidogrel, and (c) apixaban and clopidogrel standard solutions.

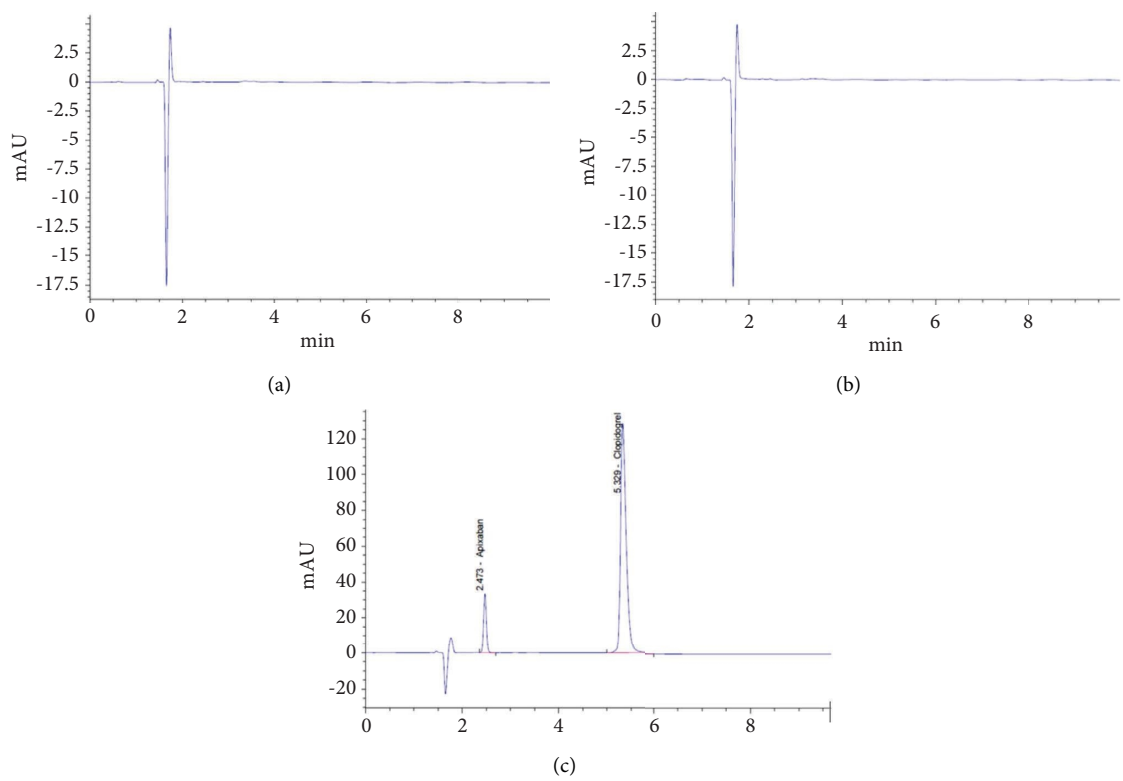
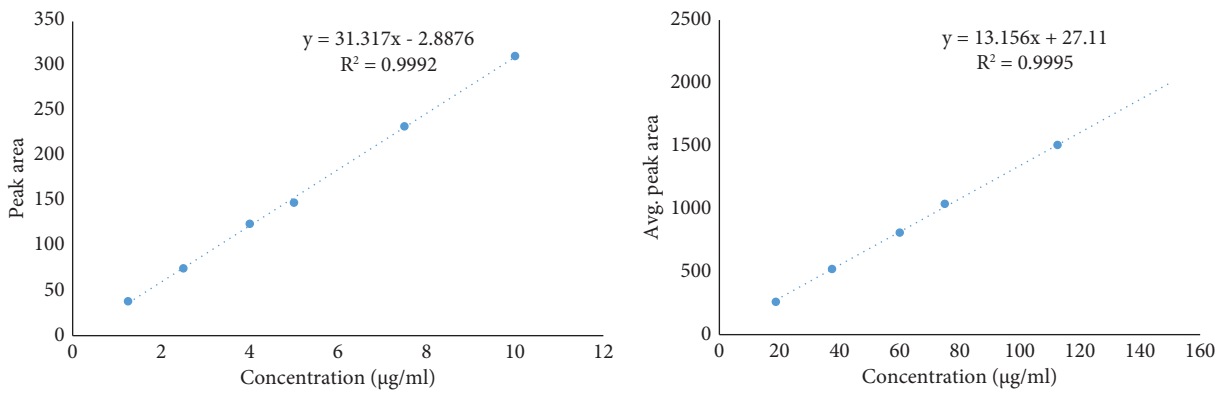


Figure 4: Specificity study chromatograms: (a) mobile phase blank, (b) placebo solution, and (c) sample solution.



(a) (b)

Figure 5: Calibration curve for linearity study: (a) APX and (b) CLOP.

Table 2: Recovery study data of the proposed HPLC method.

Actual concentration APX/CLOP (μg/ml)	Replicate number	Apixaban			Clopidogrel		
		Peak area	% recovery	Mean± SD % RSD	Peak area	% recovery	Mean± SD % RSD
2.9/35	1	87	98.974	99.708±	492	100.96	100.961±
	2	88	100.075	0.636	496	101.831	0.869
	3	88	100.075	0.64	488	100.093	0.86
5.8/75	1	175	97.935	99.036±	1029	101.54	101.371±
	2	178	99.5865	0.954	1026	101.235	0.155
	3	178	99.5865	0.96	1027	101.337	0.15
8.5/115	1	268	101.763	101.012±	1548	100.526	100.107±
	2	267	101.387	0.994	1555	100.988	1.149
	3	263	99.8847	0.98	1522	98.807	1.14

Table 3: System precision data of the proposed HPLC method.

	Injection	Peak area APX	Peak area CLOP	Rt. APX	Rt. CLOP
Standard stock with conc. 0.005/0.075 mg/mL for APX/CLOP	Inj. 1	148	1020	2.458	5.276
	Inj. 2	147	1015	2.458	5.276
	Inj. 3	147	1015	2.46	5.27
	Inj. 4	146	1021	2.46	5.269
	Inj. 5	146	1018	2.456	5.267
	Inj. 6	147	1017	2.458	5.267
	Inj. 7	146	1013	2.461	5.272
	Inj. 8	147	1013	2.458	5.267
	Inj. 9	147	1022	2.459	5.269
	Inj. 10	147	1019	2.457	5.266
Statistical analysis	Mean	146.8	1017.3	2.4585	5.2699
	SD	0.632456	3.233505	0.001509231	0.003665151
	% RSD	0.4308	0.3179	0.0613883	0.0695488
	Tailing factor	For APX	1.0587	For CLOP	1.6177
	Plate count	For APX	8568	For CLOP	10332.2
	Resolution	17.848			

Table 4: Intraday precision data and accuracy for the proposed HPLC method.

Replicate numbers	Peak area APX	Peak area CLOP	Assay for APX	Assay for CLOP
1	141	1005	96.6	99.1
2	143	1012	97.9	99.8
3	143	1018	97.9	100.4
4	142	1009	97.3	99.5
5	139	1013	95.2	99.9
6	143	1014	97.9	100
Mean ± SD	141.8 ± 1.6	1011.8 ± 4.45	97.15	99.8
% RSD	1.13	0.44		
Retention time	2.46 ± 0.002	5.27 ± 0.003		
Tailing factor	1.085 ± 0.013	1.62 ± 0.02		
Plates count	8552.7 ± 36.48	10263 ± 62.48		
Resolution	17.908			

Table 5: Interday precision data and accuracy for the proposed HPLC method.

	Peak area APX	Peak area CLOP	Assay APX	Assay CLOP
Day 1	143	1013	97.9	100.6
Day 2	147	1020	100.7	99.8
Day 3	148	1011.8	101.4	100.1
Mean ± SD	146 ± 2.65	1014.9 ± 4.43	100	99.8
% RSD	1.8	0.4		
Retention time	2.462	5.28		
Tailing factor	1.073	1.649		
Plates count	8561.67	9938		
Resolution	18.096			

Table 6: Robustness data of the proposed HPLC method.

Parameter		Retention time APX/CLOP	Resolution	Number of theoretical plates	Tailing factor	% RSD of the standard peak area
Proposed method		2.45/5.33	18.13	8662/10662	1.074/1.54	1.07/1.46
Column temperature	40°C	2.47/5.33	18.2	8520/10554	1.038/1.57	0.279/0.482
	50°C	2.44/5.2	17.8	8637/10339	1.053/1.56	0.352/0.101
Wavelength	205 nm	2.46/5.27	17.61	8585/10270	1.072/1.66	10.8/18.68
	215 nm	2.46/5.27	17.64	8597/10296	1.06/1.65	7.9/3.27
Flow rate	0.6 μL	3.67/7.9	18.98	11248/10693	1.11/1.78	22.27/22.55
	1.2 μL	1.85/3.96	16.2	6509/8850	1.08/1.53	15.37/15.11
ACN ratio	43%	2.854/6.429	18.958	9557/9797	1.056/1.764	0.352/0.735
	53%	2.23/4.569	16.211	7856/9635	1.059/1.573	1.16/0.866

Table 7: Solution stability data of the proposed HPLC method.

Solution	Retention time APX/CLOP	% RSD peak area APX/CLOP	Tailing factor APX/CLOP	Number of theoretical plates APX/CLOP	Resolution	% Recovered APX/CLOP
Standard 0	2.45/5.27	0.39/0.06	1.09//1.53	8657/10803	18.132	
48 hrs RT	2.46/5.3	0.39/0.15	1.07/1.66	8428/9772	17.515	99.5/99.9
48 hrs Ref	2.46/5.28	0.05/1	1.07/1.67	8567/9590	17.520	99.7/100.3
Sample 0	2.45/5.27	0.39/0.06	1.08/1.57	8655/10665	18.227	
48 hrs RT	2.46/5.29	0.41/0.33	1.1/1.64	8555/9637	17.535	99.5/99.9
48 hrs Ref	2.46/5.28	0.4/0.09	1.06/1.7	8580/9599	17.519	99.7/99.8

RT: room temperature, Ref: refrigerator (4°C).

a tailing factor of less than 1.5, a retention time of around 2.5 min for APX and 5.3 min for CLOP, and a resolution of around 18 (Figure 3).

3.3. Method Validation

3.3.1. Specificity. A comparison between the chromatograms of mobile phase blank, placebo solution, standard solution, and sample solution (APX. 5 μg and CLOP. 75 μg) was performed to evaluate the method specificity. As shown in Figures 3 and 4, no coeluting peaks were detected at the retention time of the two active pharmaceutical ingredients (APIs). In addition, the retention times for both APIs in the standard and the sample were found to be the same. A good resolution was observed and recorded between the APIs' peaks reflecting the absence of APIs' interference with each other. A small peak was observed before the second min. It appears in blank, placebo, and APIs chromatograms. This peak results from the eluting of TFA in the mobile phase, as its absorbance wavelength is at 210 nm [32], and a study found that a weak shoulder appears between 200 and 220 nm for TFA when added with different concentrations of ACN as a mobile phase [33].

3.3.2. Linearity, Range, and Sensitivity. The linearity of the method was determined in the concentration range of 1.25–10 μg/ml for APX (Figure 5(a)) and 18.75–150 μg for CLOP (Figure 5(b)). The linearity chromatograms for each concentration were added in the supplementary material (available here). The calibration curve was plotted using the peak area versus concentration. The correlation coefficient of APX was 0.9992, and the regression equation was $y = 31.317$, $x + 2.8876$. In addition, the correlation coefficient for CLOP was 0.9995, and the regression equation was $y = 13.156x + 27.11$.

LOD is the lowest amount of analyte that can be detected, not quantified, under the stated experimental conditions [27], while LOQ reflects the lowest amount of analyte that can be determined using the proposed method [27]. The LOD and LOQ were determined using the calibration curve method. The LOD and LOQ values were 0.3465 and 1.0499 μg/ml for APX and 3.8496 and 11.6656 μg/ml for CLOP, respectively.

3.3.3. Accuracy. The accuracy of an analytical method expresses the closeness between the values obtained by the method to the true value [27]. As shown in Table 2, the results of accuracy revealed percentage recovery at all three levels in the range of 98–102% and % RSD less than 2%, reflecting the accuracy and applicability of the proposed method for drug analysis.

3.3.4. System Precision. Ten injections were run with the proposed analytical method to carry out the system suitability study. Table 3 represents the recorded values. The % RSD was less than 2, reflecting that the system is repeatable.

Method precision results revealed that the method was precise within the acceptable limits, the % RSD for both solutions were less than 2%, the tailing factor was less than 2, and the number of theoretical plates was more than 2000, as shown in Tables 4 and 5.

3.3.5. Robustness. Robustness was tested to investigate the effect of deliberate changes in wavelength, mobile phase composition and flow rate, and column temperature on the system suitability of the proposed method. When a factor is not robust, more attention is needed to control it during the analysis method [27]. % RSD on the peak area was used to evaluate the method's robustness. Table 6 manifests a good peaks separation within the acceptable limits when changes were applied to the mobile phase composition and column temperature, whilst no robustness was achieved after applying changes on the wavelength and the mobile phase flow rate (% RSD for peak area > 2). So, more care needed to be given to control these two parameters while applying the proposed method.

3.3.6. Solutions Stability. The conducted stability study for APX and CLOP standard and sample solution at ambient room temperature and in refrigerator revealed no instability problems (Table 7).

4. Conclusion

An efficient high-performance liquid chromatography method was developed, optimized, and validated to separate the anticoagulant apixaban and antiplatelet clopidogrel in fixed-dose combination tablets according to ICH guidelines. Analysis time, resolution, and peaks' quality were optimized and evaluated. The method was found to be linear, sensitive, specific, precise, and accurate. Wavelength and mobile phase flow rate appeared to have a significant effect on robustness, so it was important to be controlled. Hence, the proposed method could be used in laboratories to determine the amount of apixaban and clopidogrel in the fixed-dose combination tablets. Globally, it is the first method developed to analyze this combination using a spectrophotometric method.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Figure 1: apixaban linearity chromatograms; A: 1.25 μg/ml, B: 2.5 μg/ml, C: 4 μg/ml, D: 5 μg/ml, E: 7.5 μg/ml, and F: 10 μg/ml. Figure 2: clopidogrel linearity chromatograms; A: 18.75 μg/ml, B: 37.5 μg/ml, C: 60 μg/ml, D: 75 μg/ml, E: 112.5 μg/ml, and F: 150 μg/ml. (*Supplementary Materials*)

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