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Master Program Industrial Pharmaceutical Technology

Formulation and evaluation of a fixed-dose combination of Apixaban and Clopidogrel in a solid oral dosage form: In vitro evaluation

تركيب جرعة فموية صلبة ثابتة من أبيكسيبان وكلوبيدوجريل وتقييمها: دراسة مخبرية

Prepared by: Ni'meh Own Ibrahim Al-Shami

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This Thesis was submitted in partial fulfillment of the requirements for the Master's Degree in Industrial Pharmaceutical Technology from the Faculty of Graduate Studies and research at Birzeit University, Palestine

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Declaration

I declared that this thesis entitled “Formulation and evaluation of a fixed-dose combination of Apixaban and Clopidogrel in a solid oral dosage form: In vitro evaluation” was submitted in partial fulfillment of the requirements for the Master's Degree in Industrial Pharmaceutical Technology from the Faculty of Graduate Studies at Birzeit University, Palestine.

I declare that the content of this thesis has been solely the result of my work, and has not been submitted for a higher degree or other qualifications.

Name: Ni’meh O. Al-Shami

Signature ……

Date: February 25, 2023

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January 30, 2023

Ni'meh O. Al-Shami.

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List of Abbreviations:

ADME: Absorption, distribution, metabolism, and elimination.

API: Active pharmaceutical ingredient.

APX: Apixaban.

BCS: Biopharmaceutical classification system.

BD: Bulk density.

CI: Carr's index.

CLOP: Clopidogrel.

CLOP-HS: Clopidogrel hydrogen sulfate.

DC: Direct compression.

DDS: Drug delivery system.

DR: Delayed release.

ER: Extended release.

ERDDS: Extended release drug delivery system.

FDA: Food and drug administration.

FDC: Fixed dose combination.

HPC: Hydroxypropyl cellulose.

HPLC: High-performance liquid chromatography.

HPMC: Hydroxypropyl methylcellulose.

HR: Hausner's ratio.

ICH: International Conference on Harmonisation.

IR: Immediate release.

MCC: Microcrystalline cellulose.

Mg. Stearate: Magnesium stearate.

MPS: Multi particulate system.

MR: Modified release.

QC: Quality control.

RLD: Reference listed drug

SLS: Sodium laurel sulfate.

SSF: Sodium stearyl fumarate.

TD: Tapped density.

TLC: Thin layer chromatography.

UHPLC: Ultra high-performance liquid chromatography.

USP: United State Pharmacopeia.

UV: Ultra-violate spectrophotometer

WG: Wet granulation.

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Abstract:

Fixed-dose combination (FDC) products represent a novel, safe, and cost-effective formulation compared to mono-product. Combined use of anticoagulant and antiplatelet medications is common among comorbid cardiovascular patients. This study aimed to formulate FDC tablets as a multidrug regimen for Apixaban 5 mg and Clopidogrel 75 mg, as prophylaxis and treatment of thrombo-embolic events.

FDC tablets were developed by combining small Immediate-Release (IR) Clopidogrel tablets with Extend-Release (ER) Apixaban tablets through direct compression and wet granulation. Further, Apixaban tablets were developed using design expert software, various types and concentrations of polymers were entered. For Clopidogrel tablets, various diluents were used to develop the IR formulation. Then, the dissolution profile for each formula was studied. Finally, the optimized formulations were encapsulated within transparent hard gelatin shell capsules to obtain fixed-dose combination tablets with two doses, Apixaban/ Clopidogrel, 5/ 75 mg, and Apixaban/ Clopidogrel, 10/ 75 mg, respectively.

All ER Apixaban formulations followed zero order and Korsmeyer-Peppas kinetics, with super case II transport mechanism as the dominant mechanism of drug release, that the release exponent “n” was more than 0.89. The apixaban drug release rate was affected by the type and concentration of the polymer used in the formulation ($P < 0.05$). As the HPMC concentration was increased, Apixaban release was retarded. For, Clopidogrel, the formulated tablets with spray dried lactose filler and sodium stearyl fumarate lubricant were found to be stable with good properties.

In conclusion, the optimum formulation yielded IR of Clopidogrel and ER of Apixaban for 24 hours with the desired in-vitro drug dissolution.

Keywords: Fixed-dose combination tablets, Apixaban, Clopidogrel, extended-release, in vitro dissolution.

ملخص الدراسة

تمثل منتجات تركيبة الجرعة الثابتة المدمجة تركيبة جديدة آمنة وفعالة وأقل تكلفة مقارنة بكل منتج على حدة. كما يعدُّ الاستخدام المشترك لمضادات التخثر والأدوية المضادة للصفيحات أمرًا شائعًا بين مرضى القلب والأوعية الدموية. هدفت هذه الدراسة لتحضير أقراص مدمجة للمادتين الفعّالتين أبيكسيبان (5 مغ) وكلوبيدوجريل (75 مغ)، كوقاية من مضاعفات تخثر الدم وعلاجه.

تم تطوير هذه الجرعة المدمجة عن طريق تصنيع أقراص صغيرة من كلوبيدوجريل ذات التحرر المباشر للدواء باستخدام تكنولوجيا الكبس المباشر للمواد، وأقراص أخرى من أبيكسيبان ذات التحرير الدوائي طويل الأمد، باستخدام تكنولوجيا التحبيب الرطب. علاوة على ذلك، طوّرت الدّراسة تركيبة أبيكسيبان باستخدام برنامج خبير التصميم، وتجربة أنواع وتراكيز مختلفة للبوليميرات. وبالنسبة لمادة كلوبيدوجريل، اعتمدت الدّراسة مواد مالئة مختلفة خلال عملية التطوير والمقارنة بين نتائجها. بعد ذلك، تم بناء نموذج لآلية تحرر الدواء من كل تركيبة ودراسته. وأخيرا، جُمِعت الأقراص المُعَدّة في كبسولات جيلاتينية شفّافة لتقديم جرعات دوائية مختلفة من كلوبيدوجريل 75 مغ/ أبيكسبان 5 مغ أو كلوبيدوجريل 75 مغ/ أبيكسيبان 10مغ. بدراسة نماذج تحرير أبيكسبان المستمر من الأقراص، وجدت الدّراسة أنّ آلية التحرر تخضع للنموذج الصفري (التحرر الدوائي الثابت غير المعتمد على تركيز المادة الفعالة) وكانت الآلية المهيمنة هي آلية النقل الفائقة II ، حيث تجاوزت قيمة "ن" لمعادلة كورسمير-بيباس (0.89). كما وُجد أن فعالية تحرر الدواء من القرص تتأثر بنوع البوليمر وتركيزه، فكانت التركيبات ذات القيم المرتفعة التركيز من البوليمر هيدروكسي بروبل ميثل سيليولوز أبطأ في تحرير الدواء منها. وفيما يتعلق بمادة كلوبيدوجريل، فإنّ التركيبة المحتوية على اللاكتوز المحضر بطريقة الرش المجفف كمادة مالئة، ومادة الصوديوم ستيريل فيوميريت كمادة مزلقة هي تركيبة مستقرة ذات صفات تحضيرية جيدة.

ختاما، وبناء على كل ما تقدّم من تجارب فقد توصّلت الدّراسة إلى تحضير كبسولة صلبة تحتوي على مادة كلوبيدوجريل الذي يذوب بشكل سريع بعد تناوله، ومادة أبيكسبان الدوائية التي يتم تحريرها بشكل مستمر مدة 24 ساعة حسب نتائج اختبار الذوبان في المختبر. الأمر الذي يؤمّل من ورائه التسهيل على المرضى في الالتزام بالجرعات الدّوائية مِمّا سينعكس بالضّرورة على الأداء العلاجي.

الكلمات المفتاحية: أقراص دوائية مدمجة، أبيكسيبان، كلوبيدوجريل، التحرر الدوائي المستمر

Chapter I: Introduction

1. Overview:

1.1. *Pharmaceutical Dosage Forms*:

A pharmaceutical dosage form is an entity administered to deliver active substances to sites of action within the body. Based on their physical form, dosage forms are classified as solid, semisolid, liquid, and gas (1). Oral drug delivery is the most convenient route of administration, as it is safe, cost-effective, flexible in design, and has high patient compliance (2). Tablets are the most common solid dosage form administered orally. These tablets are prepared by applying compression forces on powder bed or granules to formulate tablets. The compressed tablet contained active pharmaceutical agents or agents mixed with several pharmaceutical excipients used to deliver the final dosage form with specific characteristics (3). As the number of geriatrics increases globally, many are diagnosed with multiple chronic conditions or health problems which require prescribing of multiple medications (4). If the final dosage form consists of more than one API, it is called a fixed-dose combination tablet (FDC).

1.2. Fixed-Dose Combination tablets.

A Single pill combination, known as FDC, is a system formulated to deliver two or more active pharmaceutical ingredients as one unit. These dosage forms are commonly used in analgesics, cough and cold therapy, antibiotics, and multivitamins. In addition, it is found as a dosage form to treat hypertension, diabetes mellitus, respiratory disease, and HIV (5,6). The number of FDC approvals

significantly increased in the 2000s and represents an important segment of the global pharmaceutical market (7,8). Formulation of FD multidrug therapy may give a synergistic and sustained therapeutic effect associated with positive therapeutic outcomes, greater efficacy, safety improvement, and fewer side effects compared to a single maximum dose. Better patient control was also achieved due to less frequent doses and fewer missed doses (9,10). Add that patient adherence increased due to less dosing burden and lower cost (5,11).

However, these benefits are combined with potential challenges associated with FDC formulations, including drug solubility, dissolution profile, dose, possible drug interaction, and individual drug characteristics. After FDC usage other challenges may be faced as lack of inclusion in treatment protocol, less dose flexibility to fit individual medications, and the ability to determine the source of side effects (5,10).

Choosing the appropriate combination of the correct dose, route, frequency, and duration of medication based on scientific evidence is essential to improve patient outcomes and optimize medication therapy. Furthermore, inappropriate drug combinations predispose the patient to unnecessary active ingredients and potential side effects, with no real benefit for the combination as found in the Indian market(6).

Formulation of multi-drug therapy can be performed using different technologies, including monolithic, multilayer, multi-particulate, or 3D-printing systems,

depending on the compatibilities between the active constituents and the dissolution profile for each (7).

1.3. Multi particulate system (MPS):

MPS is a solid oral dosage form of active substances divided into small independent drug delivery subunits: pellets, beads, granules, mini-tablets, and multi-tablet systems (3,10).

Theoretically, MPS is an acceptable way to formulate FDC of different active substances with fewer limitations. The size of this particulate variate from micro particulate 150μm into mini tabs 3mm (12).

There are many advantages for MPS preparations that introducing multiple single units helps in formulating more than one active ingredient within the same dosage form even if they are incompatible with each other or have different dissolution profiles or different formulas, including orally dissolving particles, immediate release, and different modified release particles that control the drug release over a longer period from a single dose (12,13). Moreover, the introduction of small particles of MPS preparations with a large surface area reflects on the uniformity in gastric emptying and subsequent dissolution, which results in rapid emptying of the stomach, less toxicity, less localized irritation, enhanced drug bioavailability with higher C max at a short time, combined with uniform drug absorption and less toxicity. In addition, if the MPS preparation included a modified release system,

drug release became more identical with fewer intra and inter-individual variations, and the tendency for dose dumping became less (12,14). MPS also has the flexibility of choosing the final dosage form as a sachet, tablet, or capsule (15).

Many marketed products are prepared as MPS, such as trilipix® which is formulated as minitablets in a capsule, Rosuvastatin 5mg coated tablet, and ezetimibe 10 mg uncoated tablets which are filled in hard capsules (16), four individual multilayer diltiazem hydrochloride matrices tablets that are filled in a capsule (17), and Dutasteride/ Tamsulosine hydrochloride Teva® that is formulated as a soft capsule and pellets that filled in a hard capsule (18).

1.4. Characteristics of MPS in this study:

The common scheme of using the MPS for FDC products is to combine the IR and ER particles. In this study, the MPS was a multi-tablet system (two to three small tablets depending on dose strength) filled in a hard gelatin capsule. Tablets were prepared as ER matrix and IR drug delivery systems, using wet granulation (WG) and direct compression (DC) technologies. The final dosage form is distinguished by having the advantages of conventional compressed tablets.

1.5. Formulation technologies:

1.5.1. Direct compression (DC):

The tablet formulation is a process composed of excipients that are mixed with a pharmaceutically active agent to give the final dosage form with acceptable characteristics.

If the active agent and suitable excipients are free-flowing with good cohesiveness properties, they will be compressed directly without pretreatment to obtain perfect tablets that achieve content uniformity (19,20). Direct compression technology seems simple process with a few manufacturing steps including; weighing and blending the ingredients, adding other adjuvants such as glidant and lubricant, then forming tablets by compression (3).

DC is an efficient economic technology, with fewer manufacturing steps, less processing time, fewer labor forces, smaller equipment amount, and less process validation. Moreover, active ingredients are protected from exposure to unnecessary heat, moisture, or pressure thus increasing their stability. The resulting tablets are disintegrated rapidly, with more stable dissolution results and rapid onset of action. However, the excipient selection is a critical step as the properties of every excipient is not covered and affect the compression stage and potential difficulties expected with high dose drugs (21). These challenges added to powder handling problems and the possibility of air being entrapped among powder during the compression process, which may cause capping, splitting, or lamination (3).

1.5.2. Wet Granulation:

Granulation is a process performed to overcome powder formulation problems. The powder is known to be difficult to handle owing to its ability to segregate, agglomerate, flood, aerate or de-aerate, bridge, and arch (22).

Granules are identified as permanent agglomerated powder or enlarged particles in which the original particles can be identified (22,23). Granulation of powder performed to provide a more stable formula with denser particles, that is resist atmospheric humidity, less likely to cake or harden, freely flow, with good compressibility, less weight variation, and prevent component segregation (24). In addition, granulation enhances drugs' wettability, dissolution rate, bioavailability as well as content uniformity, and reduces dust (23,25). On the other hand, granulation requires many operational units in the process, that increases the cost and the probability of material loss during transportation. Add its inability to be used for thermos labile and moisture sensitive substances (22).

Wet granulation is a type of granulation methods that is widely used in the pharmaceutical industry (20). This process required many steps including; weighing, blending the ingredients, and adding a liquid binder to formulate a damp mass. Preparing the damp mass includes three main stages illustrated in Figure 1.1 (20);

1. Nucleation and wetting; where the liquid is distributed on powder, then engulfed to form nuclei.
2. Consolidation and growth; while the binder is still liquid, primary particles move closer to each other and collide, then larger and denser granules are formed.

3. Attrition and breakage; size reduction happens for larger and least dense granules to form several large pieces (23,26).

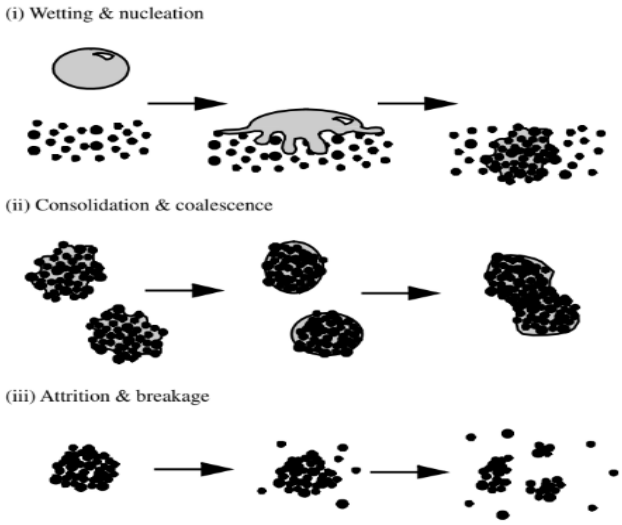

Figure 1.1: Granulation process stages

The formulated granules are screened, dried, and rescreened to size the resulting granules. Then, lubricant and blending were added, and finally, the granules were compressed to produce tablets (3). Figure 1.2 illustrates the processing steps for the two technologies. The characteristics of tablets and the drug release depend mainly on the used excipients.

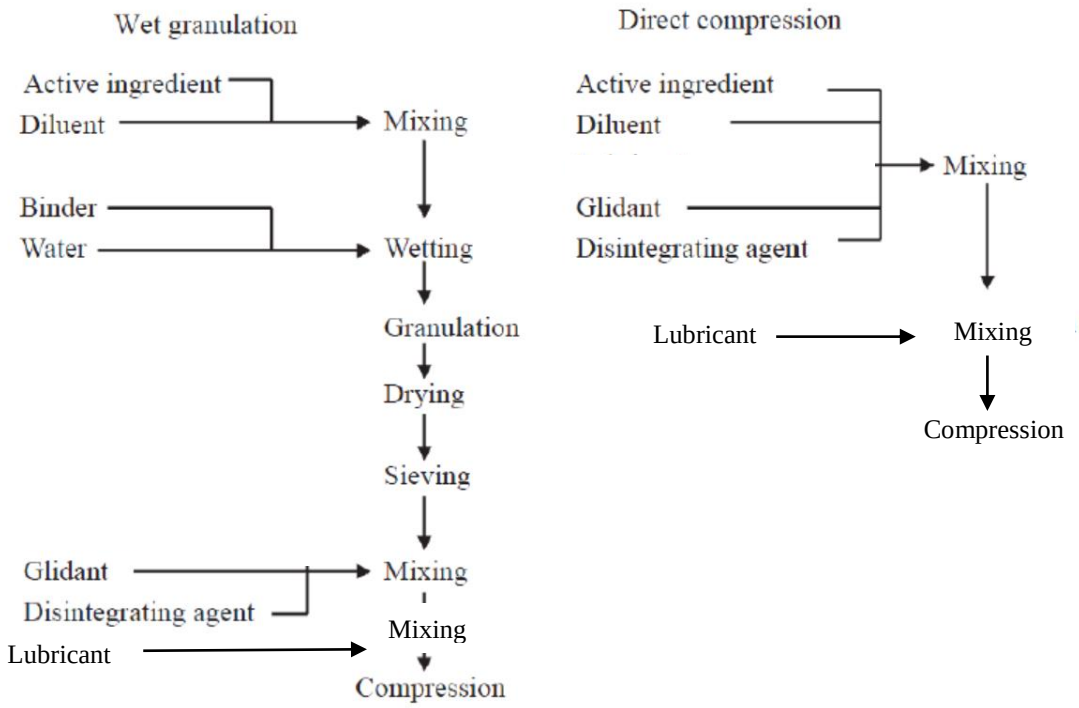

Figure 1.2: Comparison of wet granulation and direct compression processing steps

1.6. Drug Delivery System (DDS):

The drug delivery system has two main categories: immediate release (IR) and modified release dosage forms. The modified release (MR) is either an extended release (ER) or delayed release (DR) formulation.

1.6.1. Immediate release tablets:

To formulate an IR tablet, there could be a need to use diluents, binders, disintegrants, glidants, and lubricants compatible with the active agent. These excipients help to produce an integrated tablet with the desired size that is feasible to manufacture, exhibits low sensitivity to humidity and temperature, leaves no

residues in the mouth, and disintegrates rapidly upon administration (27,28). In addition, IR tablets have the merits of being suitable for manufacturing with low cost, allowing high drug loading, producing tablets with improved stability that give rapid onset of action and an expected good bioavailability, added to the possibility to provide liquid medication as a solid dosage form (28,29). However, frequent doses is expected for short half-life drug, and fluctuation of drug plasma concentration is found, which may cause side effect precipitation, and the possibility increases for drugs with a narrow therapeutic window (28). In addition, there is a need for repetition of dose within equal intervals to avoid below or over remedy (18). These constraints are faced upon using IR formulation.

1.6.2. Extended release tablets:

ER is a term used to identify a drug delivery system that is designed to release the drug continuously over a prolonged period after the administration of a single dose (30). The basic principle of this system is to apply an efficient dosage form with maximized pharmacokinetics, pharmacodynamics, and biopharmaceutical properties while reducing the side effects (31). The ER dosage forms make the drug's absorption, distribution, metabolism, and elimination (ADME) profile much more advantageous. The drug release slowed down, that protect the drug from hydrolysis and degradation in the gastrointestinal tract, minimized local side effect, and may enhance the bioavailability of some drugs. As the drug absorption is also slowed, that maintains a uniform therapeutic concentration in blood, avoiding high

blood concentration, minimizing drug accumulation with chronic doses, reducing systemic side effects and potential of toxicity became less. Among patients, less dosing frequency is expected to enhance their compliance (30,31). All these merits are faced with poor in vivo in vitro correlation, ingestion difficulties due to the large size for high doses, and high production cost.

1.6.2.1. Candidate drugs to be formulated as ER drug delivery system:

Many criteria need to be fitted by the active pharmaceutical substances to be suitable for formulation as an ERDDS. The parameter classified as physiochemical parameters and pharmacokinetics parameters.

Physiochemical parameter (32,33):

1. Molecular size: substances with large molecular size, over 500 Dalton, cannot diffuse within the polymer, so it is difficult to control the drug release from the dosage form.
2. Aqueous solubility: the aqueous solubility of the API is needed to be in the range of 0.1 - 10 mg/ ml, highly soluble and low soluble drug will be difficult to be formulated as ER dosage form.
3. Partition coefficient: Hydrophobic and too lipophilic drugs are not suitable to be formulated as ER formulas, as they will not partition into the lipid membrane, or will be entrapped and not partition out, respectively.
4. Drug pKa: Ionizable drugs are difficult to be formulated in ER dosage form, as they have a low absorption rate and less permeability.

5. Drug stability: If the drugs undergo hydrolysis or enzymatic degradation through the small intestine, they would not be candidates for ER formulation, as most of the absorption occurs there.

Pharmacokinetic parameters:

1. Elimination Half-life: drugs with a short half-life, within 2 to 8 hrs, are suitable to be formulated as ER formula, in order to avoid frequent dosing within a day.
2. Dose size: large drug doses are poor candidates; as a big dosage form will be difficult to administer.
3. Plasma concentration response relationship: if the drug's pharmacological effect is independent of plasma concentration, there is no need to formulate as an ER drug delivery system.
4. Apparent volume of distribution: Drugs with a high apparent volume of distribution are poor candidates for oral ER dosage forms.
5. Therapeutic index: Drugs with narrow therapeutic windows are unsuitable for ER formulation, as system failure or dose dumping will lead to toxicity.

1.6.2.2. Approaches to achieve an ER drug delivery system:

There are different mechanisms of drug release (Figure 1.3) (30–34);

1. Ion exchange resins: in this process, ionizable functional groups on cross-linked water-insoluble polymers form a resin-drug complex. And when appropriate ions are in contact with the ion-exchange group, the drug is released.

2. Osmosis: a constant drug release rate is achieved in this system due to the constant osmotic pressure. In this mechanism, the water is diffused to the device to compensate the solute concentration difference; the drug is released through an aqueous semipermeable membrane, then dissolved upon contact with water.
3. Dissolution: this mechanism is summarized by the detachment of the active agent from the tablet surface to the adjacent liquid interface, followed by diffusion from that interface into the bulk liquid medium.
4. Swelling: this system controls drug release via three steps; first, water diffused, then the polymer chain relaxed and the system volume increased. Finally, the incorporated drug dissolved and diffused among the relaxed surrounding polymer chain layer.
5. Erosion mechanism: the matrix size decreases due to physical, chemical, or biological reasons. Erosion is of two kinds; surface heterogeneous erosion, where only the outer part of the matrix is affected, and bulk homogeneous erosion, where the entire matrix bulk is affected.
6. Diffusion mechanism: the drug diffuses through the polymeric material, where the polymer works either as a core reservoir, and the drug diffuses through the polymeric coat, or as a matrix system.

In reality, more than one mechanism may be applied to understand how the drug is released from the DDS.

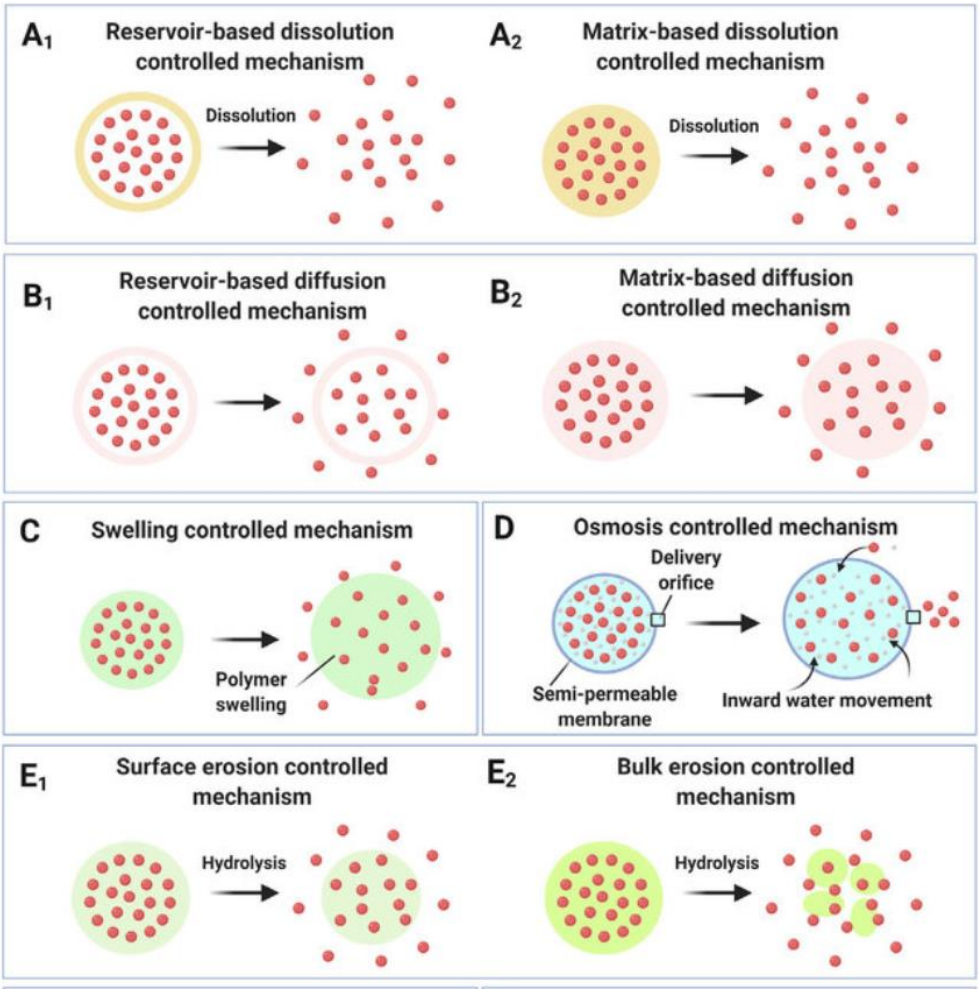

Figure 1.3: drug release mechanisms

1.7. Matrix system (33-35):

1.7.1. Overview:

Matrix tablets are the best commercial affordable sustained action drugs; as API with large doses could be loaded, the manufacturing process is easy with no previous requirements. The matrix system is composed of drug substances that are homogenously dispersed among a hydrophilic or hydrophobic matrix, which retard the drug release. This matrix can incorporate high molecular weight compounds,

added to the ERDDS advantages. On the other hand, the matrix system is dependent on the GI residence time of the dosage form, the onset of action is delayed, and the release rate is affected by food.

Different polymers may be used in matrix tablets including; hydrogels, soluble polymers, biodegradable and non-biodegradable polymers, mucoadhesive polymers, and natural gums. According to the polymer type, the produced matrix system is divided into hydrophilic lipid, hydrophobic, biodegradable, and mineral matrix.

1.7.2. Hydrophilic matrix:

This matrix is one of the most widely used, as it is a cost-effective system and obtains the desirable drug release profile. Cellulose derivatives are polymers with high gelling capacities used as a base during the formulation of matrix dosage forms. These systems are also called swellable controlled release systems (36).

1.7.2.1. Drug release mechanism from hydrophilic matrices:

After exposure of the dosage form to the aqueous media, the hydrophilic matrix rapidly hydrated, forming a gel layer, and the matrix volume increased. The outermost layer reaches a dilution point and leaves the surface of the matrix. The drug dissolved and diffused among the hydrated layer. As the water content within the polymer increased, the diffusion coefficient increased simultaneously. Deep insight on the molecular level of the polymer structure; upon hydration, the highly coiled long chain and branches start to uncoil, due to the formulation of hydrogen

bonds with water. With time, more and more hydration and uncoiling of the polymer chain occurs, and the outer region becomes weaker and leaves the polymer surface. Finally, uncoiling for the entire polymer occurs, and the whole polymer dissolved. Figure 1.4 shows these mechanisms (35,38).

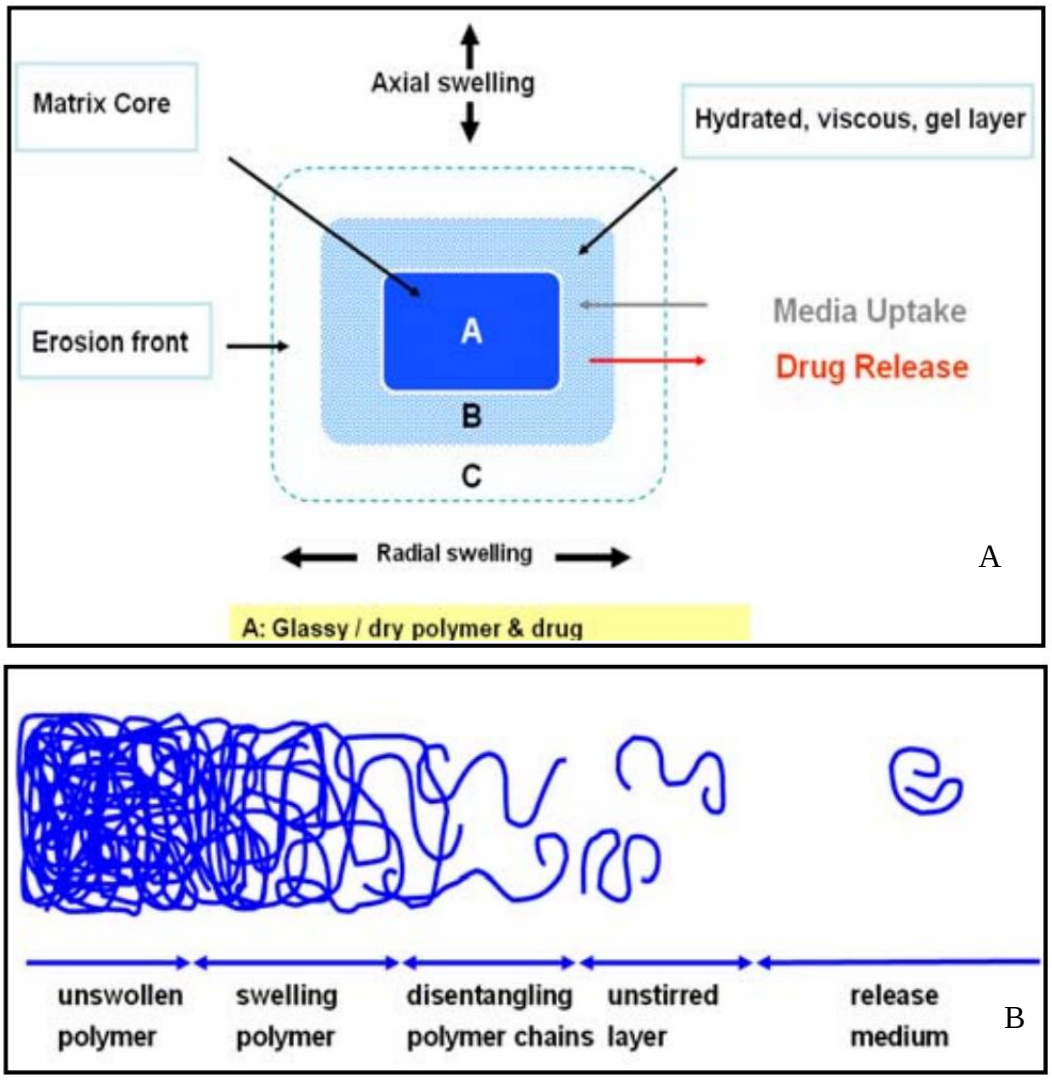

Figure 1.4: a hydrophilic matrix undergoing hydration, swelling, and dissolution. A: Tablet, B: Molecular level

1.8. Active Pharmaceutical Ingredients:

1.8.1. ***Apixaban (APX):***

1.8.1.1. Overview:

APX (Eliquis®) is an oral anticoagulant agent manufactured by Bristol-Myers Squibb S.R.L, Pfizer Limited. It is a potent, reversible, direct, and highly selective active site inhibitor of factor Xa (39,40). It prevents thrombin generation and thrombus development by inhibiting free and clot-bound factor Xa, and prothrombinase activity. 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, and the chemical formula is $C_{25}H_{25}N_5O_4$ (39,40). It is a non-hygroscopic crystalline powder, a non-ionizable compound, stable, and not sensitive to heat, light, or moisture (39). APX's aqueous solubility across the physiological pH range is ~0.04 mg/ ml, and it is classified as a BCS class III drug, a highly soluble low permeable substance according to the biopharmaceutical classification system (BCS) (40–42). APX's structure is shown in Figure 1.5 (43). This drug is administered as 5 mg twice daily and reduced to 2.5 mg in certain cases.

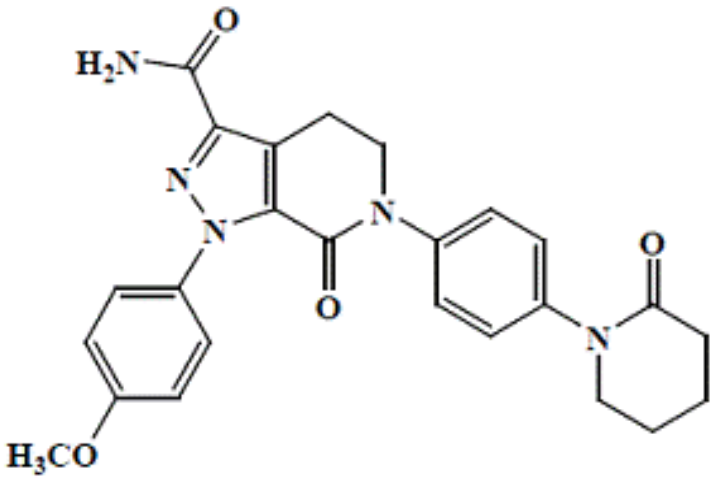

Figure 1.5: Chemical structure of Apixaban

1.8.1.2. Pharmacokinetics:

After the administration of a single dose, the absolute bioavailability is approximately 50%. Rapid absorption is achieved after oral administration and the peak is reached after 3 to 4 hours. Increasing the dose up to 25 mg is not reflected in enhancing the bioavailability of APX, and the dissolution is the control factor, due to the low solubility of APX. Around 97% of the absorbed dose is bound to plasma protein showing 21 L as the volume of distribution. Renal excretion of APX accounts for 27 % of the dose, and around 25 % was recovered as metabolites, with the majority recovered in feces (40).

1.8.1.3. Analytical Procedure:

There is no official monograph of APX in any pharmacopeia, and some studies have developed analytical methods to determine the amount of APX individually

(44–47) or in combination with other drugs (48) using high-performance liquid chromatography (HPLC).

1.8.1.4. Previous studies:

The FDA approved APX to be marketed on December 28, 2012, by Bristol-Myers Squibb/Pfizer (49), after that, many studies were performed and patents were registered to develop APX in different dosage forms for immediate release including; IR formulations for tablets or capsules (11,50,51), IR crushed tablet for oral solution (52), dispersible tablets (19), and sublingual film (53). In addition, other studies formulate APX for prolonged-release transdermal patches (54), and transdermal nano-emulsion (55), and a patent formulate it as ER tablets using only HPMC (56). APX as ER tablets are characterized to cause lower gastrointestinal irritation, reduce the bleeding risk, stabilize the drug release, and decrease administration frequencies (56).

1.8.1.5. Candidate excipients for formulation:

The choice of excipients depends on the final dosage form's intended characteristics and the inherent properties of the active agent (57). APX is a poor water soluble agent and is going to be formulated as ER formula with zero order kinetics, it is favored to be formulated as a hydrophilic matrix (34), and the candidate excipients are;

1.8.1.5.1. Hydroxypropyl methylcellulose (HPMC):

HPMC is a water soluble cellulose derivative agent, that is stable and performs a different function in the manufacturing process depending on the used grade viscosity and the added concentration during the formulation process. HPMC is used as a binder, suspending agent, film forming solution, and matrix in ER tablet formulations (57).

1.8.1.5.2. Hydroxypropyl cellulose (HPC):

HPC is also a water soluble cellulose derivative agent, it is used as a binder, film former, and an ER matrix former depending on the added concentration (57).

1.8.1.5.3. Sodium laurel sulfate (SLS):

SLS is an anionic surfactant that works as an emulsifying and solubilizing agent and has a lubricating effect (57). In addition, SLS is a candidate to be added to the formula as a wetting agent due to the inherent hydrophobicity of APX (51).

1.8.1.5.4. Magnesium stearate (Mg. St.):

This substance works as a lubricant and is used in many pharmaceutical formulations and in the Eliquis®, reflecting its compatibility with the APX (40,57).

1.8.2. Clopidogrel:

1.8.2.1. Overview:

Clopidogrel Hydrogen Sulfate (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 was synthesized as a CLOP-HS 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-HS is shown in Figure (1.6) (58). Clopidogrel is a white to off-white powder, its solubility is affected by pH, and it is freely soluble in water at pH 1. The BCS of CLOP-HS is class II, as poor soluble good permeable substances (59,60). Figure 6 shows the chemical structure of CLOP-HS salt (61).

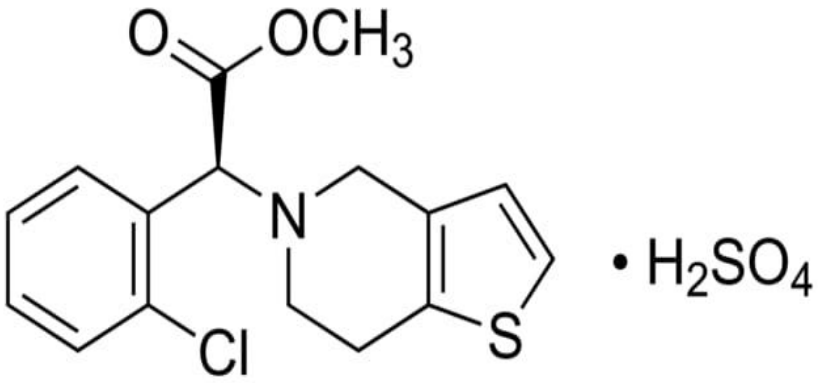

Figure 1.6: Chemical structure of Clopidogrel hydrogen sulfate.

1.8.2.2. Pharmacokinetics:

After the administration of a single dose, 50% of the dose is absorbed and binds to plasma proteins, and the peak is achieved after 45 minutes. The metabolism process takes place in the liver according to two pathways; either hydrolysis into its inactive intermediate metabolite derivative or by cytochrome p 450. Subsequent metabolism of the intermediate metabolite to form the active metabolite.

Regarding drug elimination, 50 % is excreted in the urine and 46 % in the feces 120 hours after dosing (62).

1.8.2.3. Analytical Procedure:

An official monograph published for CLOP-HS in the USP revealed that CLOP-HS could be analyzed using the HPLC method using a special column, and the absorbance is measured at 220 nm (63). In addition, many studies have aimed to develop different methods of analysis of CLOP-HS individually (64–66) or in combination using different technologies such as HPLC (67–70), Ultra high-performance liquid chromatography (UHPLC) (71), ultra-violate spectrophotometer (UV) (69), and thin layer chromatography (TLC) (72).

1.8.2.4. Previous studies:

The FDA approved Sanofi to market CLOP in 1997 (59). Many formulations were developed later in different dosage forms to enhance CLOP-HS solubility and bioavailability. A literature search revealed formulating CLOP as IR tablets (73,74), gastro retentive system as floated tablets (75), high density tablets (76) or floated osmotic capsules (77), Liquisolid compact (78,79), micro-emulsion (80), nano-suspension (81), oral disintegrating tablets (82), or fast disintegrating films (27), and as FDC system (83,84). In addition, other studies were performed to establish the compatibility between CLOP-HS and different lubricants, that magnesium stearate was found to be incompatible with CLOP-HS, while steric acid

and sodium stearyl fumarate (SSF) were found as better alternatives with less degradative effects (85–87).

1.8.2.5. Candidate excipients for formulation:

CLOP-HS will be formulated as IR tablet using DC technology, so the excipients with good flowability are candidates to be used, added to the ingredients used in the reference listed drug (RLD).

1.8.2.5.1. Mannitol:

Mannitol is a diluent with good taste. It is eligible to be used with moisture sensitive drugs like CLOP-HS. It is available as a cohesive powder and freely flowing granules. The granules have good compressibility and could be used in DC tablets (57).

1.8.2.5.2. Spray dried lactose:

Spray dried lactose is a mixture of amorphous and crystalline anhydrous lactose. Due to the manufacturing process, it can freely flow and candidate to be used as a tablet diluent that is prepared via DC technology (57).

1.8.2.5.3. Microcrystalline cellulose (MCC) 112:

MCC is a well-known diluent used in manufacturing tablets using different technologies. MCC grade 112 has the characteristics of a larger mean particle size and low moisture content (<1.5%), which makes it suitable for use as a diluent with a moisture-sensitive API formulated using DC technology. Increasing the particle size enhances the flow properties of the substances (57).

1.8.2.5.4. Crospovidone:

Crospovidone is a super disintegrant agent used to facilitate the disintegration process for tablets prepared either by DC or granulation methods. It also acts as a solubilizing agent and enhances the solubility of poorly soluble drugs (57). CLOP-HS classifies as BCS class II, and using Crospovidone may facilitate the tablet disintegration and enhances the drug solubility.

1.8.2.5.5. Sodium stearyl fumarate (SSF):

Mg. St. is found to be incompatible with CLOP-HS, as it causes a degradation effect (85). SSF is a candidate to be used as a lubricant instead of Mg. Stearate for tablets and capsules, but in higher concentrations as it is found to be not as effective as Mg. Stearate, and found to be more compatible with CLOP-HS (57,85).

1.9.Evaluation:

Evaluation of the formulation process is required to investigate the physical and chemical properties of a drug substance and the resulting formula. The evaluation is performed on the powder blend and the final dosage form. These tests generate valuable information that helps formulate an acceptable, safe, efficacious, and stable product.

Evaluation of blend:

1.9.1. Evaluation of blends:

To characterize the final blend flow and compressibility, angle of repose and bulk/ tapped density are two tests carried out to determine the blend flowability. Carr's

Index (CI%) and Hausner's ratio (HR) are calculations performed to determine the blend flow. These test procedures are explained in the USP General Chapters <616> and <1174> (88,89). The calculations were performed according to the following equations:

The angle of repose is calculated according to the following equation;

$$Tan\,\Theta = \frac{h}{r}$$

where (h) and (r) are the cone's height and radius.

The following equation is used to calculate bulk density.

$$BD = \frac{M}{V0}$$

where M= blend mass and V0= blend bulk volume before tapping.

The tapped density equation is:

$$TD = \frac{M}{Vt}$$

where M= blend mass and Vt= blend final tapping volume.

Carr’s Index

$$\%\,CI = 100\,\frac{TD - BD}{TD}$$

Hausner’s ratio

$$HR = \frac{V0}{Vt}$$

The flow characteristics are determined based on the CI and HR values listed in Table 1.1.

Table 1.1: Effect of Carr's Index and Hausner's Ratio on flow property:

Carr's Index	Flow character	Hausner's Ratio
<10	Excellent	1.0- 1.11
11-15	Good	1.12- 1.18
16- 20	Fair	1.19- 1.25
21- 25	Passable	1.26- 1.34
26- 31	Poor	1.35- 1.45
32- 37	Very poor	1.46- 1.59
>38	Very, very poor	>1.6

1.9.2. Physical evaluation of FDC tablets:

1.9.2.1. Weight variation and tablet thickness:

This quality control (QC) test is applied to distinguish the dosage form uniformity. It is applied to solid dosage forms, where the active pharmaceutical ingredient compromises approximately 25 % of the dosage form by weight (90).

1.9.2.2. Hardness test:

This test is done to determine the tablets' resistance to crushing and their ability to withstand mechanical shocks while manufacturing, packaging, and shipping (91,92).

1.9.2.3. Friability test:

This test is performed to evaluate the durability of the tablets during packaging and shipping. The test calculates the mass lost under stress conditions. The tablets were observed if any crack, split, or break occurred, and reweighed to calculate the percent weight loss. A wider sense of fragmentation and shipping is included in the

test (92,93). The friability value for each formula was calculated according to the following equation:

$$\%\ friability = 100\ \frac{Winitial - W\ final}{W\ initial}$$

1.9.2.4. In vitro disintegration:

This test is performed to determine whether the dosage form disintegrated within a predetermined period when placed in liquid media. Disintegration is not related to the dissolution of the active constituents (94).

1.9.2.5. Assay:

The assay is a quality control test that helps determine the drug's product potency. Furthermore, it ensures that the formulated dosage form contains the needed % amount of active substances (92).

1.9.2.6.In vitro drug release study:

1.9.2.6.1. In vitro dissolution studies:

Dissolution studies were carried out under standardized conditions to evaluate drug performance, but did not definitively demonstrate bioavailability. This study determines the rate of mass transfer from a solid dosage form surface into the dissolution media (95,96).

1.9.2.6.2. Kinetic analysis of dissolution data:

Scientists have reviewed the dissolution kinetics and developed mathematical models describing the dissolution mechanism (95). These models include;

Zero-order kinetics refers to constant drug release over time, which is not affected by the drug amount in the dosage form. This model can be presented by the following equation:

$$Q_t = Q_0 + K_0 t$$

Where Q_t= amount of drug released at time t, Q_0= initial amount of drug in the tablet, K_0= zero order drug release constant.

The first order model reflects the dependence on the concentration gradient difference between the solid surface layer and the bulk liquid. This concept is similar to the diffusion model, and is represented using the following equation:

$$\log Q_t = \log Q_0 + \left(\frac{Kt}{2.303}\right)$$

where Q_t = amount of drug released at time t. Q_0= initial amount of drug in the tablet, K= first order drug release constant.

Higuchi model is a model that tried to refer the release rate of a drug to the physical constants based on laws of diffusion, and the equation is:

$$Q_t = K_H t^{\frac{1}{2}}$$

where K_H= Higuchi diffusion constant, Q_t= amount of drug dissolved at time t.

In addition, the Hixon-Crowell model that used to describe a system with a change in the dosage form surface or diameter. It can be presented by the following equation:

$$Q_0^{\frac{1}{3}} - Q_t^{\frac{1}{3}} = K_{HC}\mathrm{t}$$

t; where Q_t = amount of drug released at time t. Q_0= initial amount of drug in the tablet, K_{HC}= Hixon-Crowell drug release constant.

Finally, the Korsmeyer–Peppas model has derived a simple relationship to describe the drug release from the polymeric system. This model considers the simultaneous occurrence of water diffusion, tablet swelling and gel formation, drug and filler diffusion, and the dissolution of the polymeric matrix. The model can be presented by the following equation:

$$\log\frac{M_t}{M_0} = \log K_m + n\log t$$

where log M_t/ M_0 = fraction of drug released at time t, K_m = rate constant, n = release exponent and its value interoperates the drug release mechanism related to the geometrical shape of the delivery system. That $n \leq 0.45$ indicates Fickian diffusion; $0.45 < n < 0.89$ indicates anomalous diffusion; n= 0.89 indicates case II transport, and $n > 0.89$ indicates super case II transport mechanism. Anomalous diffusion refers to diffusion and erosion controlled drug release, while case II transport and super case II transport refer to the erosion mechanism (95).

1.9.7. *Swelling and erosion studies:*

These studies are conducted to understand the polymer behavior and drug release mechanism under dissolution conditions.

1.9.8. *Stability study*:

Stability studies are performed to provide evidence on how the quality of the drug is affected with time by environmental conditions such as humidity, temperature, and light. It also helps in identifying the recommended storage condition and the shelf life of the dosage form. International Conference on Harmonisation (ICH) Q1A (R2) guideline was published to identify the stability testing types, conditions, and sampling time (96).

Chapter II: Study Objectives

2. Study importance:

2.1. *Clinical overview:*

Cardiovascular disease (CVD) is a major cause of morbidity and mortality globally, consequently, the rising cost of health care. The yearly number of new cases increased and the total number of prevalent cases doubled between 1990 -2019. Add that the total number of disability-adjusted life years (DALYs) had increased, and the number of death steadily increased within that period (97). Yearly, it takes approximately 17.9 million lives, and in the United States, it is classified as the leading cause of death among men and women (98), while over three-quarters of deaths are reported in middle and low-income countries (99). Acute coronary syndrome (ACS) is a symptomatic type of CVD that is responsible for one-third of the deaths of people older than 35 (100,101). Atrial fibrillation is another disease that affects the elderly, it is a risk for ischemic diseases and heart failure. Its prevalence increased by 33 % within the last 20 years and its expected burden would exceed 60 % by 2050, and the incidence is higher among developed countries (99,101). Anti-thrombotic agents are used in single, dual, or triple therapy based on patient diagnosis and risk factors as prophylaxis for cardiovascular events and complications. However, using multi-antithrombotic agents simultaneously increases the risk of bleeding, as the main side effect, and hospitalization. Many reviews were performed to evaluate these regimens and revealed that CLOP is the preferable agent from the P2Y12 receptor antagonist due to its lower adverse

medication effect of bleeding. Furthermore, in a clinical trial, dual therapy, including CLOP and APX, was superior to triple therapy with Aspirin or dual therapy with warfarin due to reduced medication-related reversed effects and less hospitalization (102–107).

Regarding APX, patients are administered a dose of 5 mg twice a day. Clinical studies were performed to assess the blood concentration after administration of an IR tablet of 10 mg once a day and revealed that more fluctuation in blood concentration was observed compared to a dose of 5 mg twice a day. So it is favored to be administered twice a day (108,109).

2.2. Study objective:

The present study aimed to develop novel FDC tablets of multi-tablet system and evaluate their in vitro dissolution profile. The FDC consisted of one CLOP-HS (98 mg) tablet and one or two APX (5 mg each) tablets, matrix type, encapsulated within translucent gelatin shell capsules (0 size).

The study also aimed to develop a simple, rapid, and accurate HPLC analytical method for quantifying the amount of APX and CLOP-HS in a novel FDC dosage form. The method was validated according to ICH guidelines, by assessing its specificity, linearity, precision, accuracy, limit of detection, limit of quantification, and robustness.

Chapter III: Method of analysis

3. HPLC method development and validation.

Developing an accurate analytical method is a crucial step during the development process of a new formula. A new FDC of APX and CLOP was developed and no analytical method was developed before. This chapter presents a simple, rapid, and accurate HPLC analytical method that was developed to quantify the amount of APX and CLOP in a novel FDC dosage form. The method was validated according to ICH guidelines, by assessing its specificity, linearity, precision, accuracy, limit of detection, limit of quantification, and robustness.

3.1. Materials and methods

3.1.1. Materials and Reagents:

A pharmaceutical grade of APX and CLP-HS were donated by Pharmacare PLC (Palestine). HPLC grade of Acetonitrile (ACN), Trifluoroacetic acid (TFA), and Triethylamine (TEA) were purchased from Merck (Merck Serono Amman, Jordan). Water was obtained by filtration using a cellulose nitrate filter (0.45) micron manufactured by Sartorius stedim biotech company (Jordan). Tablet excipients including HPMC, 28-30% methoxyl, 7-12% Hydroxypropyl, viscosity (2% aq. soln., 20^{o}C) 7500-14000 mPa.s and HPC were purchased from Alfa Aesar (by Thermos Fisher Scientific, United Kingdom). Methocel, SLS, spray dried lactose, mannitol, MCC 112, Colloidal silica, SSF, Mg stearate, and anhydrous ethanol were donated from Pharmacare PLC (Palestine).

3.1.2. 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 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, sonicator, and vacuum filter pump were also used during the analysis.

3.1.3. Selection of wavelength:

Five mg of APX and 98 mg of CLOP-HS (equivalent to 75mg CLOP) were dissolved in 100 ml of ACN. From this solution, 5 ml was obtained and diluted in a 50 ml volumetric flask using purified water (PW) to give standards with 0.005 and 0.075 mg/ ml of APX and CLOP, respectively. A blank was prepared by mixing ACN: PW, 5:50 (v/v). A scan for the wavelength with a good absorbance was performed between 200-400 nm.

3.1.4. Chromatographic conditions:

The buffer was prepared by dissolving 0.5 ml of TFA in 1000 ml of PW, 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 ^{o}C.

3.1.5. Preparation of stock and standard solutions:

A stock solution with a concentration of (0.05 and 0.75 mg/ ml) APX and CLOP was prepared by weighing 5 mg of APX, and 98 mg of CLOP-HS equivalent to 75 mg CLOP, dissolved in 100 ml HPLC grade ACN. Then sonicated for 5 min. to obtain a standard solution of a mixture of APX and CLOP.

3.1.6. Preparation of sample solutions:

Ten FDC (APX and CLOP-HS) tablets were weighed, placed in a mortar, and finely powdered. The tablet powder equivalent to 5 mg APX and 75 mg CLOP were transferred into a 100 ml volumetric flask. About 40 ml of ACN were 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 Clarify® syringe filters of 0.45 µm. From this sample stock solution, 5 ml were transferred into a 50 ml volumetric flask. The volume was made up to the mark with PW. The prepared solution was injected into the HPLC to obtain the APX and CLOP content percentage in the tablets.

3.1.7. Preparation of placebo solution:

The placebo solution for the FDC tablets was prepared by mixing all the excipients (HPMC, HPC, Methocel, SLS, spray dried lactose, Colloidal silica, SSF, and Mg. Stearate in ACN, then 5 ml were transferred into 50 ml volumetric flask, and volume was completed by water.

3.1.8. Method Validation:

The proposed analytical method was validated concerning parameters such as specificity, linearity, range, accuracy, precision, detection limit, quantification limit, and robustness as described in ICH guidelines Q2 (R1) (110), and the FDA guidance (111).

3.1.8.1. 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 (110). The specificity of the method was determined by injecting blank, placebo, standard, and sample solutions separately and recording the chromatograms using the proposed method.

3.1.8.2. 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 PW. Next, the solutions were injected in triplicate into an HPLC column. Then, calibration curves were plotted against the final concentrations, and the regression equations were obtained.

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

$$LOD = 3.3\ \sigma/S$$

$$LOQ = 10\ \sigma/S$$

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

3.1.8.3. Accuracy:

The accuracy was evaluated by a recovery study. 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.

3.1.8.4. 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. As well to assure method precision, intra, and inter-day studies were carried out. Intra- day precision was studied by analyzing six replicates of prepared samples of tablets preparation within the same day. The inter-day 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 inter-day studies.

3.1.8.5. 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 drug solutions. 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.

3.1.8.6. 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.

3.2. Results and discussion:

3.2.1. Selection of wavelength:

The absorption spectra of APX and CLOP showed a good absorbance detected at a wavelength around 210 and 223 nm (Figure 3.1). The selected as λ max was 210

nm for the combination therapy as the mixture of drugs showed significant absorbance and fewer noises at the baseline of HPLC spectra.

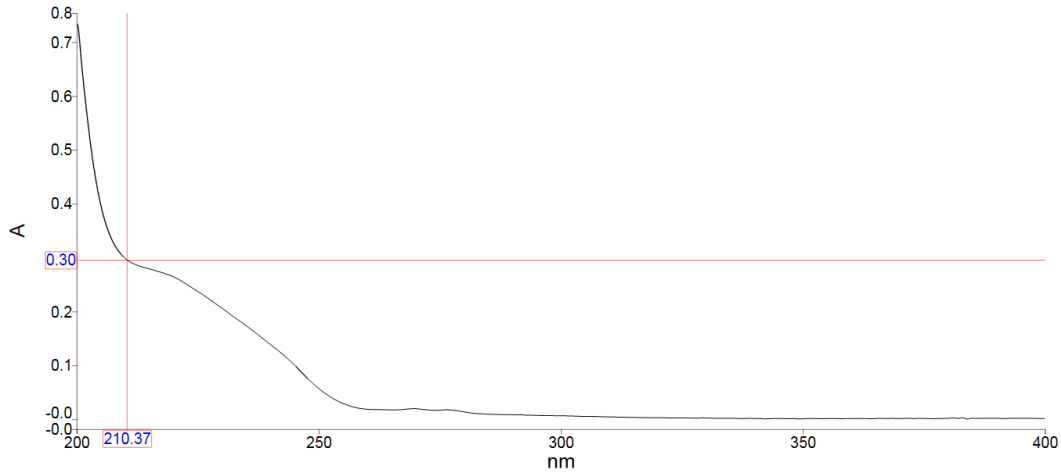

Figure 3.1: UV spectra of APX and CLOP standards in 40ACN:60 PW

3.2.2. 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 column type, mobile phase type, ratio, pH, flow rate, column temperature, wavelength, and injection volume. Table 3.1 summarized 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), PH (2.2). The injection volume was 5 µL, the mobile phase flow rate was 0.9 ml/ min., the column temperature was set to 45 ^{o}C, at wavelength λ 210 nm, and the run time was set on 10 min. The resulting

peaks were eluted forming two sharp peaks, almost symmetric in shape with a tailing factor of less than 1.5, with a retention time of around 2.5 min for APX and 5.3 min for CLOP, and a resolution of around 18 (Figure 3.2).

Table 3.1: Chromatographic condition scanning trials

Mobile phase %(v/v)	Flow rate (ml/min)	Injection volume (µL)	Wavelength (nm)	Column temp. (^{0}C)	Mobile phase pH	Observation	Result
PW.: ACN, 60:40	1	20	205	Ambient	NM.	No peaks	Rejected
PW.: ACN, 60:40	1	20	224	Ambient	NM.	No peaks	Rejected
PW.: ACN, 40:60	1	20	224	Ambient	NM.	1 peak	Rejected
PW.: 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 & 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, 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

PW.: Purified water, ACN.: Acetonitrile, PB.: Phosphate buffer, TFA.: Triflouroacetic acid buffer, TF.: Tailing factor, Abs.: Absorption, NM.: not measured.

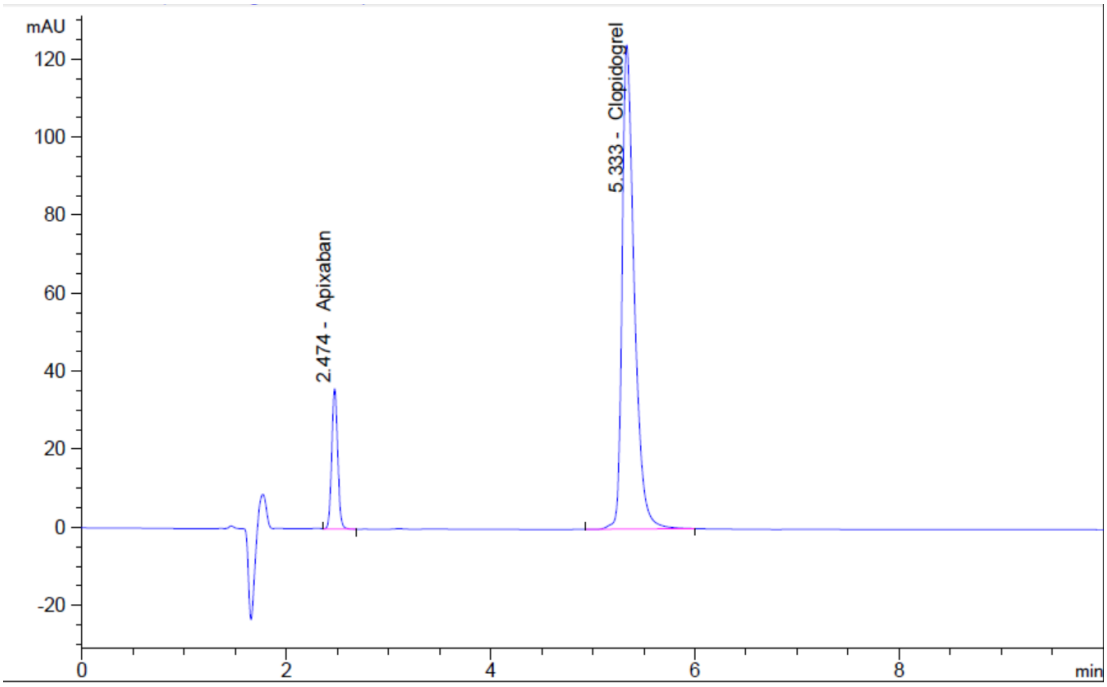

Figure 3.2: Chromatogram of APX. & CLOP. standard solution.

3.2.3. Method validation:

3.2.3.1. Specificity:

A comparison between the chromatograms of mobile phase blank, placebo solution, standard solution, and sample solution (APX. 5µg and CLOP. 75µg) was performed to evaluate the method specificity. As shown in Figures (3.2 & 3.3), no coeluting peaks were detected at the retention time of the two APIs. In addition, the retention times for both APIs in standard and sample were found to be the same. A good resolution was observed and recorded for the APIs peaks reflecting the absence of APIs interference with each other.

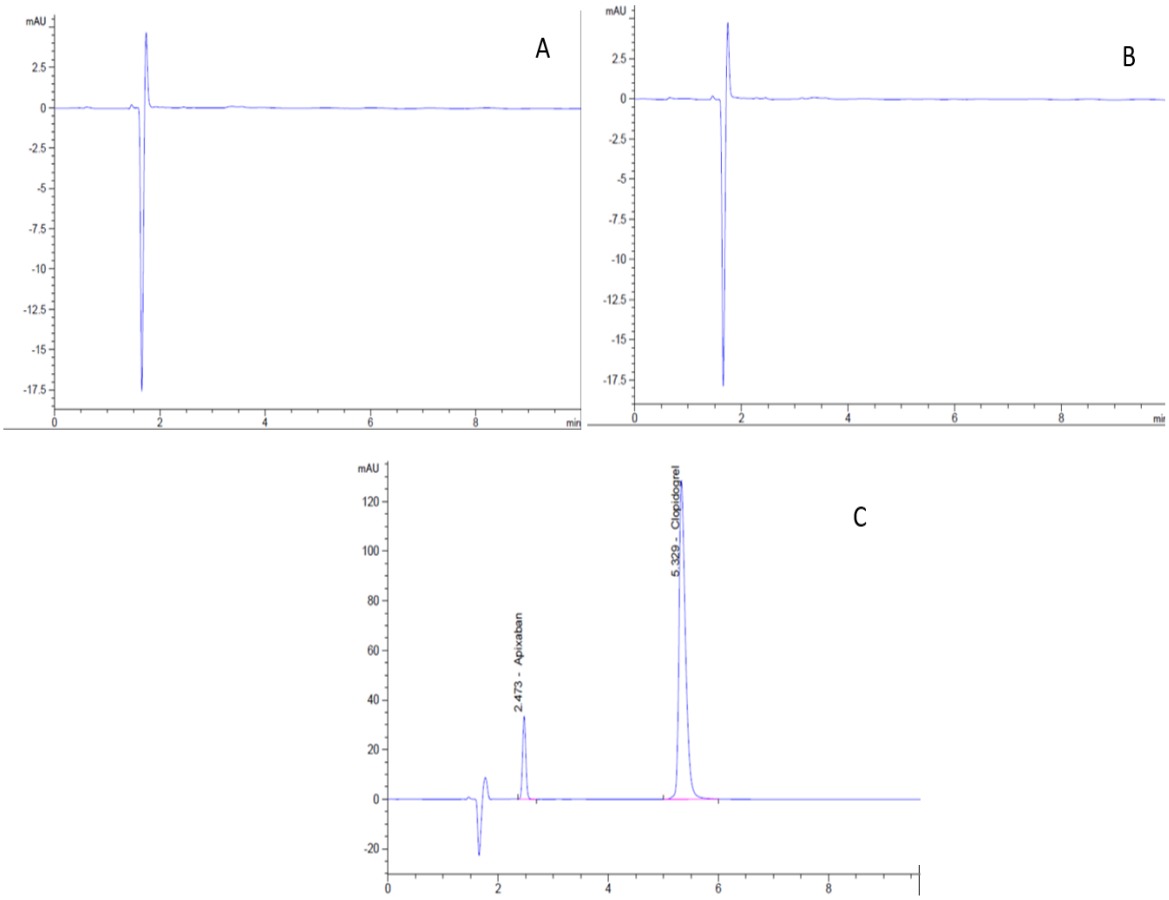

Figure 3.3: Specificity study chromatograms: A mobile phase blank, B: Placebo solution, C: Sample solution.

3.2.3.2. Linearity, range, and sensitivity:

The linearity of the method was determined in the concentration range of 1.25 - 10μg/ml for APX. (Figure 3.4.A), and 18.75- 150μg for CLOP (Figure 3.4.B). The calibration curve was plotted using the peak area versus concentration. The correlation coefficient of APX was found to be 0.9992, and the regression equation was (y = 31.317x + 2.8876). Add that 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 (110). While LOQ reflects the lowest amount of analyte that can be determined using the proposed method (110). The LOD and LOQ were determined using the calibration curve method. The LOD and LOQ values were 0.3465 and 1.0499μg/ ml for APX, and 3.8496 and 11.6656μg/ ml for CLOP, respectively.

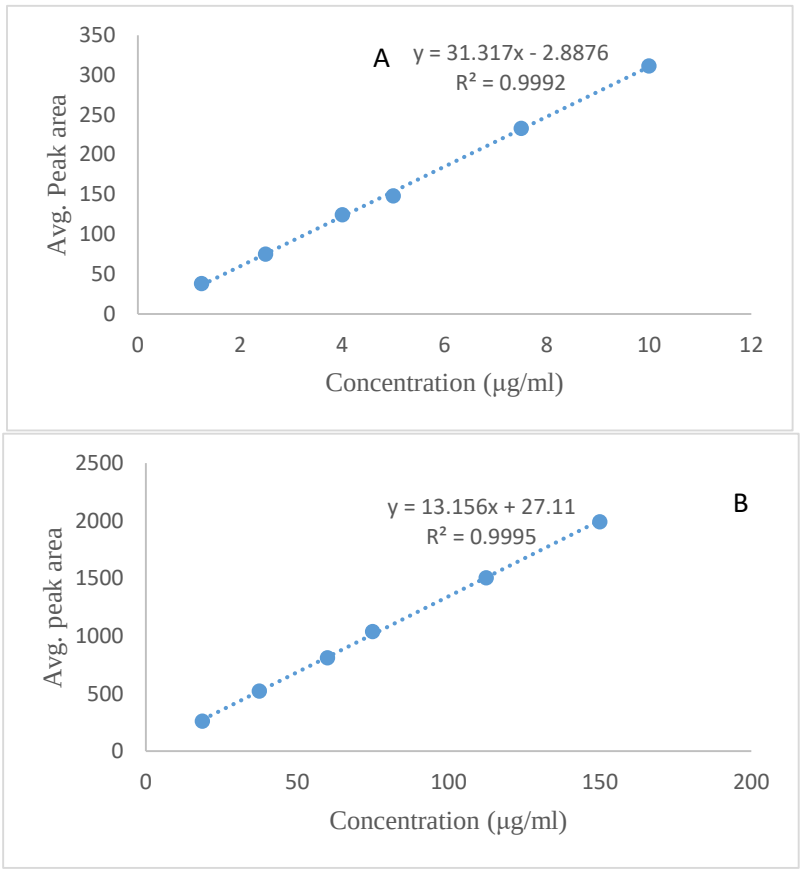

Figure 3.4: Calibration curve for linearity study, A: APX, B: CLOP.

3.2.3.3. Accuracy:

The accuracy of an analytical method expresses the closeness between the values obtained by the method to the true value (110). As shown in table 3.2, 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.

Table 2.2: 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± 0.636 0.64	492	100.96	100.961± 0.869 0.86
	2	88	100.075		496	101.831	
	3	88	100.075		488	100.093	
5.8/75	1	175	97.935	99.036± 0.954 0.96	1029	101.54	101.371± 0.155 0.15
	2	178	99.5865		1026	101.235	
	3	178	99.5865		1027	101.337	
8.5/115	1	268	101.763	101.012± 0.994 0.98	1548	100.526	100.107± 1.149 1.14
	2	267	101.387		1555	100.988	
	3	263	99.8847		1522	98.807	

3.2.3.4. System Precision:

Ten injections were run with the proposed analytical method to carry out the system suitability study. Table 3.3 represents the recorded values. The % RSD was less than 2, reflecting that the system is repeatable.

Table 3.3: 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/ 0.075mg/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			

Method precision results revealed that the method was precise within the acceptable limits, that 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 3.4 & 3.5.

Table 3.4: Intra-day precision data and accuracy for the proposed HPLC method:

Replicate Number	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 3.5: Inter-day 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			

3.2.3.5. 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 (110). % RSD on peak area was used to evaluate the method's robustness. Table 3.6 manifested a good peaks separation within the acceptable limits when changes were applied to the mobile phase composition and column temperature. While 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.

Table 3.6: Robustness data of the proposed HPLC method

Parameter		**Retention time APX/CLOP**	**Resolution**	**Number of theoretical plates**	**Tailing factor**	**%RSD of standard peak area**
Proposed method		2.45/5.33	18.13	8662/10662	1.074/1.54	1.07/1.46
Column temp.	40ºC	2.47/5.33	18.2	8520/10554	1.038/1.57	0.279/0.482
	50ºC	2.44/5.2	17.8	8637/10339	1.053/1.56	0.352/0.101
Wavelength	205nm	2.46/5.27	17.61	8585/10270	1.072/1.66	10.8/18.68
	215nm	2.46/5.27	17.64	8597/10296	1.06/1.65	7.9/3.27
Flow rate	0.6µL	3.67/7.9	18.98	11248/10693	1.11/1.78	22.27/22.55
	1.2µL	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

3.2.3.6. 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 3.7).

Table 3.7: 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 ^{o}C).

Chapter IV: FDC development and evaluation

4. FDC development and evaluation

4.1. overview:

An FDC is a system formulated to deliver two or more active pharmaceutical ingredients as one single unit and there is a global orientation toward FDC production. APX and CLOP are antithrombotic agents recommended for AF patients who had ACS and had undergone percutaneous coronary intervention (PCI). This chapter presents the development method and in vitro evaluation of a novel FDC of ER APX tablets and IR CLOP-HS tablets as a multi-tablet system. This combination is not available as a final dosage form in the markets, and no previous published studies prepared this formula before.

4.2. Materials and methods:

4.2.1 Materials and reagents:

A pharmaceutical grade APX and CLOP-HS were received as gifts from Pharmacare PLC (Palestine). Tablet excipients including HPMC, 28-30% methoxyl, 7-12% Hydroxypropyl, viscosity (2% aq. soln., 20^{o}C) 7500-14000 mPa.s HPC was purchased from Alfa Aesar (by Thermos Fisher Scientific, United Kingdom). Methocel, SLS, spray dried lactose, mannitol, MCC 112, Colloidal silica, SSF, Mg stearate, and anhydrous ethanol were donated from Pharmacare PLC (Palestine). For buffer preparation, Sodium hydroxide pellets, Sodium phosphate tribasic dodecahydrate, 98%, and hydrochloric acid were analytical

grades and purchased from Carlo Erba Reagents, Acros organics, and Merck Serono, respectively. Translucent hard gelatin shell capsules (0 size) were donated from Jerusalem Pharmaceuticals Co. Ltd. (Palestine).

4.2.2. Instruments:

APX and CLOP-HS tablets were formulated on a manual single-punch tablet compression machine. Pharma test® (Germany) instruments were used to perform friability, hardness, disintegration, and dissolution tests. Copley® tapped density tester, Ohaus® electronic balance, Ohaus® LOD tester, pH/orp meter, manual sieves, digital caliper, Elma® bath sonicator, and DHG- 9023A dry oven were used during formulation and analysis. Compatibility study experiments were conducted using a Bruker FT-IR vacuum spectrometer equipped with a Platinum ATR unit with single reflection diamond crystal (Bruker Optik GmbH, Rosenheim, Germany), and the obtained spectra were compared using OPUS viewer software. For stability studies, samples were kept in a climate chamber (BINDER, Tuttlingen, Germany).

4.2.3. Compatibility study:

Drugs excipients compatibility study was conducted to evaluate the compatibility of CLOP-HS with APX and the compatibility of each API with pharmaceutical excipients expected to be used in the formulation process (112,113). A binary mixture of API and each excipient was prepared in a 1:1 ratio and stored in a

stability chamber at room temperature and at 40 ± 2 ºC/ 75 ± 2 % RH for four weeks, while other samples were stored in a dry oven at 60 ºC for two weeks. The samples were tested for their physical appearance, and their compatibility was evaluated using Fourier Transform Infrared Spectroscopy (FT-IR). The FT-IR peak matching method spectrum was used for compassion. The samples were scanned in the wavelength range of 4000- 400 cm^{-1}.

4.2.4. Tablets formulation:

4.2.4.1. Preparation of Clopidogrel tablets:

The proposed formulas are listed in (Table 4.1). CLOP-HS tablets were formulated using DC technology. All ingredients were weighed for 150 tablets per batch for each of the three proposed formulas. All ingredients, except glidant and lubricant, were blended manually for 5 min using a mortar and a pestle. The glidant was then added and blended manually in a polypropylene bag for 2 min, followed by the lubricant, which was added and blended manually in a polypropylene bag for 2 min. Finally, the blends were directly compressed with a manual single-punch tablet machine using 6 mm biconcave punches.

Table 4.1: Clopidogrel formulated batches.

Ingredients	Function	Formulation code (mg/ tablet)		
		CLOP 1	CLOP 2	CLOP 3
Clopidogrel hydrogen sulfate	API	98	98	98
Lactose spray dried	Diluent	42.9	0	0
Mannitol	Diluent	0	42.9	0
MCC 112	Diluent	0	0	42.9
Klucel	Binder and disintegrant	7.5	7.5	7.5
Sodium stearyl fumarate	Lubricant	1.5	1.5	1.5
Colloidal silica	Glidant	0.15	0.15	0.15
Total		150.05	150.05	150.05

4.2.4.2. Preparation of Apixaban tablets:

4.2.4.2.1. Formulation development:

APX formulation development aimed to obtain stable sustained-release tablets that were completely released within 24 h.

Based on the excipients compatibility studies and revising the reference listed drug (RLD) product, different laboratory scale experiments were prepared and tested to obtain the appropriate dissolution profile. The experiment included various manufacturing technologies, including DC, WG with water, and absolute ethanol. Table 4.2 shows the tested formulas' composition and their manufacturing process. Dissolution experiments were performed on three tablets of each formula to discriminate between formulas and identify the minimum and maximum amounts of excipients required to produce tablets with the intended characteristics. Eleven formulas were prepared until we clearly understood the excipients' behavior and the appropriate manufacturing process.

Table 4.2: Formulation trials of Apixaban extended release tablets:

Ingredient	ER 1	ER 2	ER 3	ER 4	ER 5	ER 6	ER 7	ER 8	ER 9	ER 10	ER 11
Apixaban	5	5	5	5	5	5	5	5	5	5	5
HPMC	35	35	35	20	25	50	0	0	20	20	20
HPMC E5	0	0	0	0	0	0	20	50	30	0	25
HPC	35	35	35	30	45	0	30	0	0	0	25
Avicel 101	0	0	0	20	0	20	20	20	20	50	0
SLS	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mg stearate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	75.75 mg										
Manufacturing process	D.C*	WG. PW**	WG. Et-OH***								

*D.C.: direct compression, ** WG. PW: wet granulation with purified water.
***WG. Et-OH: wet granulation with absolute ethanol.

4.2.4.2.2. Factorial design:

For the determined excipients, a full factorial design (2^4), D-optimal level, was employed using design expert software version 6.0.4.1 (STAT- EASE), and 4 factors were evaluated. One replicate was run, and then the experimental trials were performed at the sixteen resulting combinations. Combinations of HPMC (X1), HPC (X2), Methocel E5 (X3), and SLS (X4) were selected as independent variables (Table 4.3). The mean dissolution time (MDT), $T_{25\%}$, and $T_{90\%}$ were selected as dependent variables. The data were subjected to 3D response surface methodology to determine the influence of the three polymers and SLS on the dependent variables.

All the formulations contained the same quantities of APX and Mg. Stearate (5 mg and 0.025, respectively), but varying quantities of polymers and SLS, and the total tablet weight was 75.75 mg (Table 4.4). APX ER tablets were formulated by WG technology. All excipients were weighed separately for 150 tablets per batch for

each formula. First, excipients, except SLS and Mg stearate, were added in the geometric method. Next, polymers and APX were blended manually for 5 min in a mortar and a pestle, and absolute ethanol was sprayed to formulate granules. The formulated granules passed through mesh size #12, dried in a dry oven at 40 ^{o}C for 30 min, then crossed into mesh size #16. Finally, SLS and lubricant were manually added and blended in a polypropylene bag for 2 min. The final blend was compressed with a manual single-punch tablet machine using 6 mm biconcave punches.

Table 4.3: Experimental design plan generated by software:

Component	Code	Minimum	Maximum	Coded low	Coded High
A. HPMC	X 1	20	35	+0	+0.495868
B. Methocel E5	X 2	0.25	25	+0	+0.818182
C. HPC	X 3	20	35	+0	+0.495868
D. SLS	X 4	0	0.25	+0	+0.00826446

Table 4.4: Ingredients of Apixaban ER tablets based on factorial design (APX1- APX16):

Formula code	Ingredient (mg/ tab.)						
	Apixaban	HPMC	Methocel E5	HPC	SLS	Mg stearate	Total
APX 1	5	35	0.5	35	0	0.25	75.75
APX 2	5	27.5	7.87	35	0.12	0.25	75.74
APX 3	5	35	0.25	35	0.25	0.25	75.75
APX 4	5	20	15.5	35	0	0.25	75.75
APX 5	5	20	15.25	35	0.25	0.25	75.75
APX 6	5	20	25	25.37	0.12	0.25	75.74
APX 7	5	25.5	25	20	0	0.25	75.75
APX 8	5	27.05	16.15	27.05	0.25	0.25	75.75
APX 9	5	25.25	25	20	0.25	0.25	75.75
APX 10	5	35	15.37	20	0.12	0.25	75.74
APX 11	5	35	7.87	27.5	0.12	0.25	75.74
APX 12	5	30.12	20.12	20	0.25	0.25	75.74
APX 13	5	20	20.12	30.12	0.25	0.25	75.74
APX 14	5	31.04	12.05	27.29	0.12	0.25	75.75
APX 15	5	24.85	20.61	24.85	0.19	0.25	75.75
APX 16	5	25.5	25	20	0	0.25	75.75

The final Fixed-dose combination (FDC) consisted of one CLOP-HS (98mg) tablet and one or two APX (5mg each) tablets encapsulated within translucent hard gelatin shell capsules (0 size).

4.2.5. Evaluation of blends:

Various parameters, such as angle of repose and bulk/ tapped density, were determined to characterize the final blend flow and compressibility. In addition, Carr's Index (CI%) and Hausner's ratio (HR) were also calculated as per USP General Chapter <616> and <1174> (88,89).

4.2.6. Evaluation of FDC tablets:

4.2.6.1 Weight variation and tablet thickness:

Ten tablets were randomly selected for each of the 16 formulas of the APX and CLOP-HS batch. First, the means and standard deviations were calculated. The official limit of percentage deviation is 10 % for APX, as the average tablet weight is less than 130 mg, and 7.5 % for CLOP-HS, as the average tablet weight lies in the range between 130 - 324 mg. Then, a digital caliper scale was used to determine the thickness of the tablets, and the average thickness was calculated.

4.2.6.2. Hardness test:

Tablet hardness was determined using a Pharma-test hardness tester. The test was carried out on 10 randomly selected tablets from each of the 16 batches of APX and

the optimized CLOP-HS batch. First, the hardness was measured in Kilo Pascal (Kp), then the mean and the standard deviation were calculated.

4.2.6.3. Friability test:

Friability was obtained with a pharma-test friabilator at 25 rpm for 4 min on a 6.5 g weight of tablets, as the average weight of the individual tablet is less than 650 mg. The weighed tablets were placed in the drum, and the test ran. Then, the tablets were observed if any crack, split, or break occurred and reweighed to calculate the percent weight loss.

4.2.6.4. In vitro disintegration:

The disintegration times of the IR CLOP-HS formulated tablets were evaluated using a pharm-test disintegrator that operated at 37 ± 2 ^{o}C. Six tablets of CLOP-HS were placed into the six cells of the rack, one per cell, and then immersed in water. The time required for complete disintegration was recorded, and the mean time was calculated.

4.2.6.5. Assay:

Ten tablets of each formula were powdered in a mortar and pestle. The weight of one tablet (75.75 mg and 150.05 mg of APX and CLOP-HS, respectively) was dissolved in 100 ml ACN, left over a night to ensure complete dissolution, 5 ml of solution was filtered in a 50 ml volumetric flask through a 0.45 μm syringe filter,

and the volume was made up using purified water. The drug content assay was evaluated by the HPLC developed method.

4.2.6.6. In vitro drug release study:

4.2.6.6.1. In vitro dissolution studies:

For CLOP-HS, in vitro drug release studies were carried out in 750 ml of 0.1 N HCL for 2 hrs using a USP type II dissolution apparatus (paddle type) at 75 rpm and 37 ± 0.5 ^{o}C. For APX, in vitro, drug release studies were carried out in 900 ml of 0.05 M Sodium phosphate buffer with 0.05 % SLS for 24 hrs using a USP type II dissolution apparatus (paddle type) at 75 rpm and 37 ± 0.5 ^{o}C.

The in vitro drug release study for the final FDC, two strengths, was carried out using the USP delayed release method A (114). The study was performed in 750 ml of 0.1 N HCL using a USP type II dissolution apparatus (paddle type) at 75 rpm and 37 ± 0.5 ^{o}C for the first 2 hrs. Samples were withdrawn at (5, 10, 20, 30, 45, 60, and 120 min.). After 2 hrs, 250 ml of 0.2 M tribasic sodium phosphate equilibrate 37 ± 0.5 ^{o}C was added to the dissolution media, and the pH of the media was adjusted to (6.8 ± 0.05). The apparatus continued to run for 24 hrs. A sample of 5 ml was withdrawn from the dissolution media in a specified period (4, 8, 12, 16, 20, and 24 hrs.).

All dissolution studies were performed on three tablets of each formula. While the dissolution for the final optimum formula was carried out on six tablets. A sample of 5 ml was withdrawn from the dissolution media using an auto-sampler, and no

volume correction was made. The samples were filtered using a 0.45 μm Clarify® syringe filter. The absorbance of the samples was measured using a spectrophotometric method at 210 nm using HPLC developed method and the % cumulative release (% CR) was plotted using calculated mean values of cumulative drug release versus time.

4.2.6.6.2. Kinetic analysis of dissolution data:

The release data were fitted to five kinetic models including; zero-order, first-order, Higuchi, Hixon-Crowell, and Korsmeyer-Peppas to determine the drug release mechanism with the aid of DD Solver add-in software. The drug release mechanism was considered according to the coefficient of determination; R^2 values (34).

4.2.6.7. Swelling and erosion studies:

The swelling nature of tablets was studied by water gain on 3 tablets. The swelling index study was performed using USP dissolution apparatus-II in the final dissolution media at 37 ± 0.5 ºC, rotated at 75 rpm. At predetermined intervals for 24 hrs, the tablets were withdrawn using a predetermined weight mesh, and excess dissolution media was removed with absorbent tissue, then weighed. The percentage swelling of the tablet was determined according to the following equation:

$$\%\ \text{Water uptake} = 100\ (W_t - W_0)\ /\ W_0$$

where W_t is the mass in the swollen state at time t, and W_0 is the initial tablet weight.

The matrix erosion was determined on the same tablets, at the same time intervals. After weighing the hydrated tablets, they were dried in an oven at 60 ^{o}C for 24 hrs., and the remaining dried were weighed. The percentage erosion (% mass loss) was determined according to the following equation:

$$\%\text{Erosion} = 100(W_0 - W_r) \,/\, W_0$$

Where W_o was the initial tablet weight, W_r was the dry weight after time t.

The remaining percentage was determined using the following equation:

$$\%\, Dried\ remaining = 100 - \%\, erosion$$

4.2.7. Stability study:

Forty capsules of the optimized batch were packed in a screw-capped amber glass bottle and kept for accelerated stability study at 40 ^{o}C/ 75 % R.H. in a climate chamber (BINDER, Tuttlingen, Germany) for 90 days. Accelerated stability study samples were analyzed at 0 and 90 days for physical appearance and drug content concerning the initial results of the same batch.

4.2.8. Statistical analysis:

To investigate the significance of the differences between the results from the studied formulations, a one-way analysis of variance (ANOVA) test was used. The significance level was set at ($\alpha < 0.05$). Design Expert software version 6.0.4.1 (STAT- EASE) was used for analysis.

4.3. results and discussion:

4.3.1. *Compatibility study:*

The most suitable excipients are chosen based on the literature and RLD product search to be suitable and fulfill the purpose of the study. The FT-IR spectra were recorded for pure API and powder mixtures of API and each excipient to assess any possible chemical interactions between API and the excipients (115). The FT-IR spectra were studied at zero time and after exposure to different stress conditions as defined by ICH guidelines (96). Table 4.5 reflects the FT-IR peak values (cm^{-1}) for each functional group. Figure 4.1 demonstrates the FT-IR spectra of pure APX, CLOP-HS, and their physical mixture upon preparation and after exposure to stress conditions, Figures 4.2 and 4.3 show each API with the physical mixture with each excipient. No physical changes were observed, and the obtained spectra for pure APIs correlated well with that of the APIs mixture and with excipients and showed all the characteristic peaks with no major changes. Hence, no significant chemical interaction occurs in the solid state at zero time. In addition, no deviations were observed between peaks of each mixture at zero time and after exposure to stress conditions. This finding concludes that the APX and CLOP-HS are compatible with each other, and each API was compatible with its formulation components.

Table 4.5: FT-IR data of APX and CLOP-HS.

API	Functional groups	IR values (cm^{-1})
Apixaban	N-H stretch	3482
	N-H stretch	3308
	C-H stretch	2902
	C=O stretch	1679
Clopidogrel	N-H stretch	2501
	C-S-C stretch	2345
	C=O stretch	1751
	C=C stretch	1438
	C-O stretch	1062, 1152, 1185

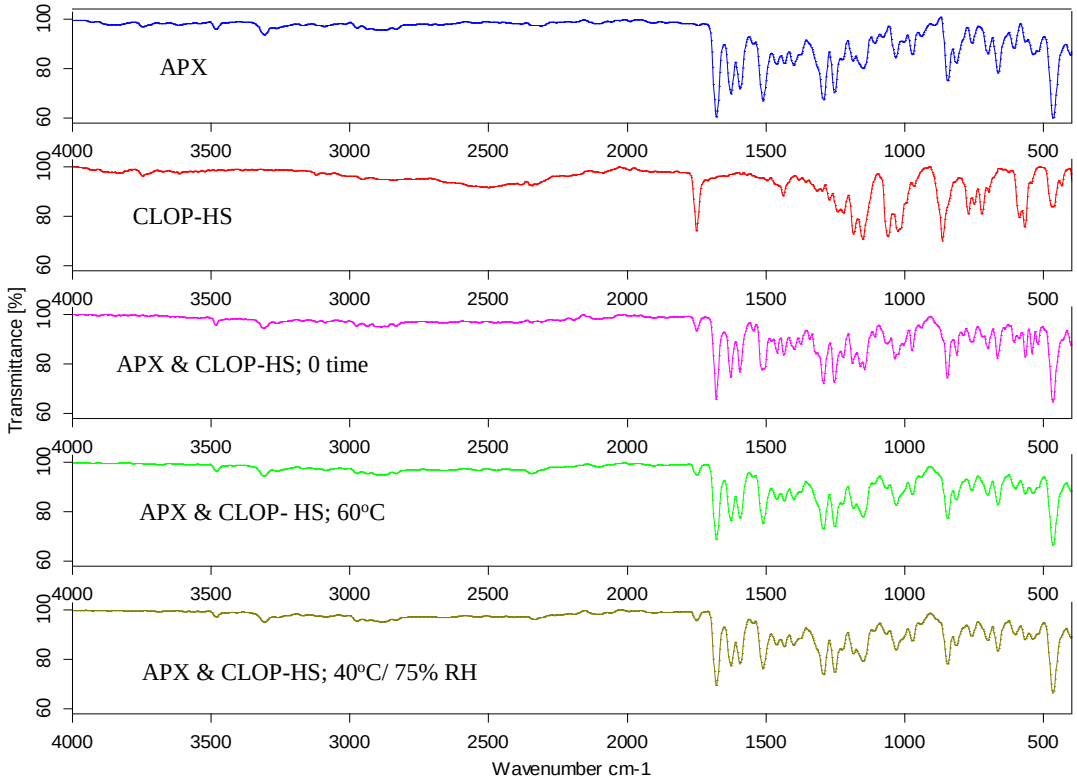

Figure 4.1: FT-IR spectra of pure APIs and their physical mixture in different conditions.

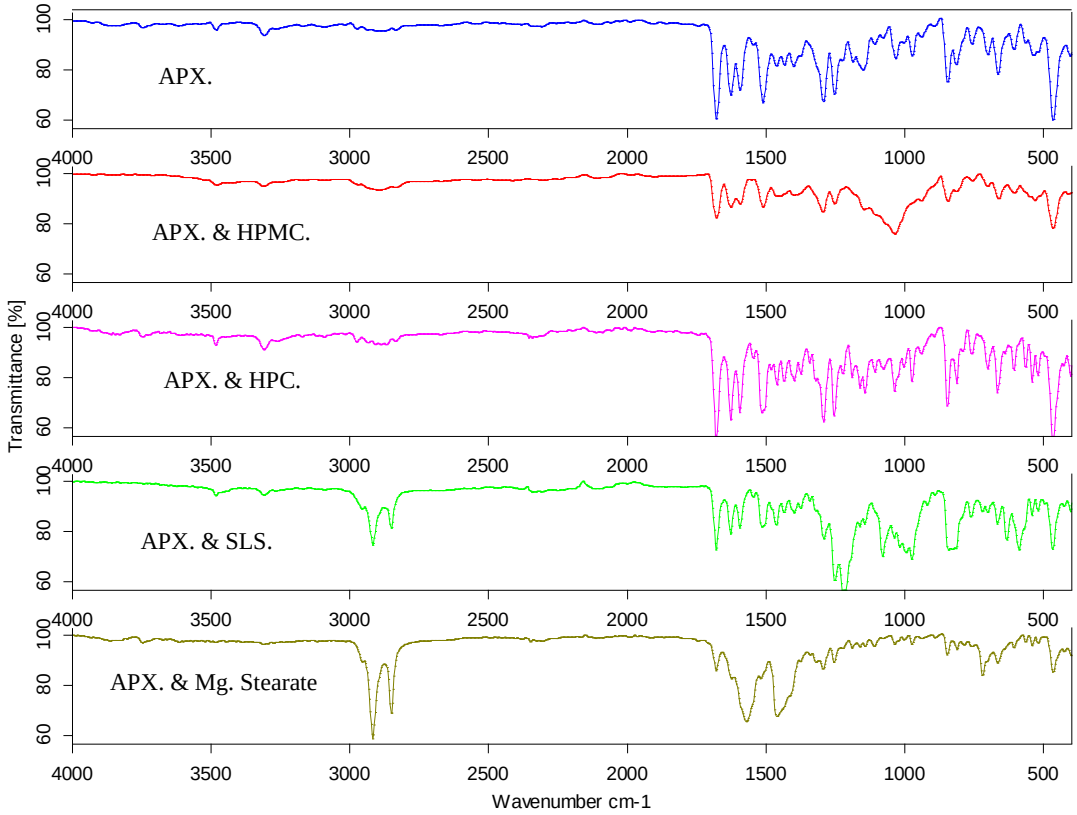

Figure 4.2: FT-IR spectra of pure APX. and a physical mixture of APX. with each excipient. APX.: Apixaban, HPMC: Hydroxypropyl methylcellulose, HPC.: Hydroxypropyl cellulose, SLS.: Sodium lauryl sulfate, Mg. ST.: Magnesium Stearate.

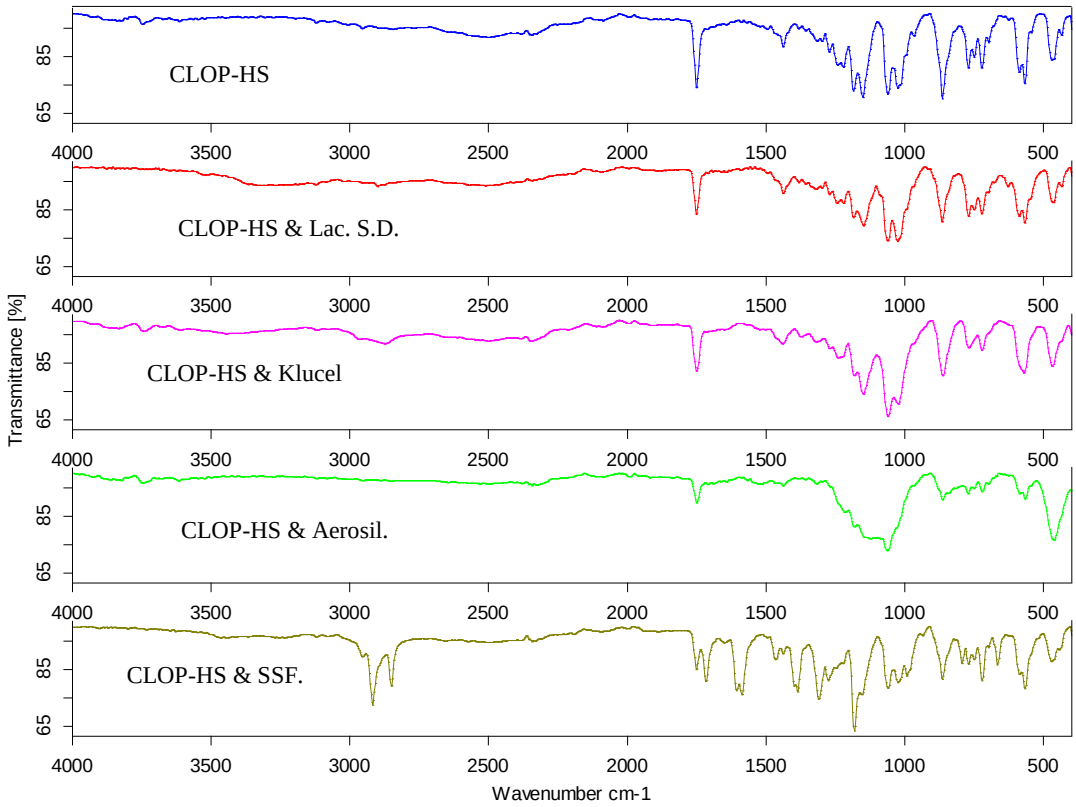

Figure 4.3: FT-IR spectra of pure CLOP-HS. and a physical mixture of CLOP-HS. with each excipient.
CLOP-HS.: Clopidogrel Hydrogen sulfate, Lac. S.D.: spray dried lactose, Aerosil: Colloidal silica, SSF.: Sodium stearyl fumarate.

4.3.2. Formulation development:

4.3.2.1 Development of IR CLOP-HS tablets:

Three formulations of CLOP-HS (CLOP-HS 1- CLOP-HS 3) were formulated using different diluents including mannitol, MCC 112, and spray dried lactose. Observation of the characteristics of the powder bed showed that the formula with mannitol (CLOP-HS 2) had a higher potential to stick with punches during the compression process, while the formula with MCC 112 (CLOP-HS 3)

showed a longer disintegration time (within 10 min) compared to CLOP-HS 1 and CLOP-HS 2 were disintegrated within 4 and 7 min, respectively. The dissolution test results revealed that the CLOP-HS 1 and 2 were released completely (% CR> 96%) within the first 30 min of the test, while 76% of CLOP-HS 3 was released within the first 30 min. Since CLOP-HS is formulated via direct compression, using mannitol powder enhances the stickiness tendency (116), and using MCC retard disintegration and dissolution due to higher binding properties and hydrophobicity (117) compared to mannitol and lactose, the formula with spray dried lactose (CLOP-HS1) was chosen as an optimum formula to scale up.

4.3.2.2. Development of ER APX tablets:

As preliminary experiments, eleven APX formulas were investigated (ER 1 - ER 11), with each formula containing different quantities and types of polymers, as well as different manufacturing technology.

The first three formulations (ER 1- ER 3) were prepared by direct compression, wet granulation using purified water, and wet granulation with absolute ethanol, respectively.

The DC formula (ER 1) was found to have poor content uniformity, and its assay was 76 %. While the WG formula (ER 2) with purified water poured by dropper

produced large and sticky wet granules, they hardened after drying and could not be sieved or compacted into tablets. The produced granules remained sticky and difficult to handle when the experiment was repeated using a spraying nozzle. The third formula (ER 3) was prepared with absolute ethanol that was sprayed on the powder bed and mixed with a spatula, the formulated granules were fluffier than ER 2 and have accepted characteristics, they were non-sticky, easy to handle, and the granulation end point was determined easily.

As APX has low compressibility, low flowability (11), low dose, and is prepared with hygroscopic polymers; wet granulation technology was chosen. It aids in the preparation of a more stable formula that is less likely to cake or harden, has improved flowability and compression characteristics, decreased weight variation and loss of blended powder quality, enhanced drugs wettability, bioavailability as well as content uniformity (23,24).

Subsequently, different polymer ratios were prepared using the same technology (ER 4 – ER 11), and the behavior of the resulting tablets was evaluated according to their dissolution profiles. The primary findings were that increasing the HPMC ratio resulted in APX release extending longer than 24 hrs, while increasing the Methocel E5 ratio resulted in tablets that disintegrated upon exposure to dissolution media. Increasing the HPC ratio accelerated the drug release to less than 24 hrs. Increasing HPC level indicates the predominance of swelling, which enhances the chance of diffusion over erosion (118). While increasing the quantity of HPMC

resulted in the rapid formation of a strong, thick, and turbid gel layer, that resists water diffusion and surface erosion process; and that retard water uptake, drug diffusion, or release (119,120). The quantities of polymers that give formulas with extended release within 24 hrs were determined to be within the range of 20 to 35 mg for HPMC and HPC, and less than 25 mg for Methocel E5. Furthermore, several Apixaban ER formulas (APX 1 – APX 16) were developed and evaluated utilizing these findings and a factorial approach.

4.3.3. Evaluation of blend:

Table 4.6 presents the findings of various evaluations of the blend characteristics investigation for formulation APX 1 - 16. The results of bulk densities for all formulations of APX were in the range of 0.19 - 0.35 g/ ml and 0.33 g/ ml for CLOP-HS. The findings of tapped density for APX formulations were in the range of 0.24 - 0.40 g/ ml and 0.38 g/ ml for CLOP-HS. The angle of repose values were between 29º - 41º for APX and 34º for CLOP-HS. Carr's Index and Hausner's ratio were between 7.1 - 17.6 % and 1.0 - 1.2, respectively for APX, and 13.3 % and 1.15 for CLOP-HS. These results revealed excellent to good flow for all the formulas of APX except formulas APX 8, which showed fair flow and APX 4 which showed passable flow. That is related to the manufacturing process as the formulation was prepared in the wet granulation method (23). Add that CLOP-HS showed good flow even the bad flowability of CLOP-HS using spray dried lactose. The spray dried

process was known to yield spherical lactose particles with uniform size and flow easily (121), and that reflects on the flowability of the final blend.

These results indicated that all blends have acceptable flow properties, compressibility, and all pre-compression parameters were within the acceptable ranges.

***Table 4.6: Evaluation of blend characteristics (n=3)*.**

Formulation code	Bulk Density	Tapped density	Angle of repose Ɵ	Flow Property	Carr's index	Hausner's ratio	Scale of flowability
APX 1	0.31	0.333846	32	Good	7.142857	1.076923	Excellent
APX 2	0.250915	0.294825	35	Good	14.89362	1.175	Good
APX 3	0.246926	0.272122	35	Good	9.259259	1.102041	Excellent
APX 4	0.282214	0.348618	41	Passable	19.04762	1.235294	Fair
APX 5	0.278918	0.310614	35	Good	10.20408	1.113636	Excellent
APX 6	0.305957	0.343268	34	Good	10.86957	1.121951	Good
APX 7	0.357852	0.402583	32	Good	11.11111	1.125	Good
APX 8	0.297844	0.339316	39	Fair	12.22222	1.139241	Good
APX 9	0.302717	0.343827	31	Good	11.95652	1.135802	Good
APX 10	0.300867	0.347154	29	Excellent	13.33333	1.153846	Good
APX 11	0.2462	0.284077	32	Good	13.33333	1.153846	Good
APX 12	0.335605	0.390027	31	Good	13.95349	1.162162	Good
APX 13	0.310667	0.362444	35	Good	14.28571	1.166667	Good
APX 14	0.198118	0.240571	30	Excellent	17.64706	1.214286	Fair
APX 15	0.3032	0.36384	30	Excellent	16.66667	1.2	Fair
APX 16	0.331286	0.3865	31	Good	14.28571	1.166667	Good
CLOP-HS 1	0.3267	0.3769	34	Good	13.3333	1.1538	Good

4.3.4. Evaluation of tablets:

The evaluation of the resulting tablets of the sixteen APX formulations and CLOP-HS formulation is presented in table 4.7. Among APX and CLOP-HS batches the results revealed that all batches showed a total weight loss of less than 1 % after the

friability test, had a uniform tablet thickness, whereas the hardness of tablets was variate in the range of 4.13 - 5.7 kp for APX formulations. The hardness and thickness values showed sufficient mechanical resistance in all the patches. Furthermore, a disintegration test was performed on IR CLOP-HS1 tablets, and the disintegration time range was between 3 - 4 min, within the accepted limits. For the assay test, APX tablets assay were between 95 - 102 %, which indicates getting accuracy in dosing. All evaluation parameter values were within the acceptable limits according to the USP 38-NF 33 and European Pharmacopoeia.

Table 4.7: Evaluation of formulation batches of tablets.

Formula code	Weight variation	Thickness	Hardness	Friability (%loss)	Moisture content (%LOD)	Assay (%)
APX 1	76.09±2.9	2.99±0.07	5.69±0.49	0.06	4.68	95.33
APX 2	75.5±2.4	2.99±0.07	5.3±0.416	0.097	3.95	95.72
APX 3	75.8±2.37	2.97±0.07	5.03±0.37	0.04	4.12	97.04
APX 4	75.18±2.11	2.92±0.06	4.75±0.26	0.6	4.26	96.1
APX 5	75.04±2.15	2.89±0.06	4.82±0.43	0.2	3.99	99.36
APX 6	75.81±2.19	2.92±0.07	4.76±0.43	0.4	3.84	100.89
APX 7	75.31±2.12	2.92±0.07	4.22±0.3	0.4	3.51	101
APX 8	74.47±2.08	2.9±0.05	4.13±0.5	0.02	3.9	98.22
APX 9	75.38±1.72	2.9±0.08	4.92±0.15	0.22	3.99	99.64
APX 10	74.49±2.4	2.89±0.08	4.77±0.31	0.14	4.05	101.76
APX 11	75.48±2.26	2.92±0.06	5.38±0.85	0.04	4.43	95.77
APX 12	76.49±1.86	2.95±0.07	4.48±0.31	0.16	3.85	98.76
APX 13	76.52±2.19	2.9±0.08	4.89±0.45	0.18	3.39	96.21
APX 14	75.69±3.13	2.91±0.09	5.29±0.47	0.03	4.64	95.15
APX 15	75.51±2.64	2.92±0.07	4.42±0.36	0.04	4.65	95.08
APX 16	76.86±2.27	3.02±0.04	4.65±0.42	0.09	4.12	102.23
CLOP-HS 1	148.9±3.56	4.36±0.08	6.63±0.61	0.65	2.9	99.8

The upper limit is 83.3mg for APX, and 161.3 for CLOP-HS.
The lower limit is 68.2mg for APX, and 138.8 for CLOP-HS.

4.3.5. Experimental design and response surface analysis:

A full factorial design 2^4 was selected as it helps in understanding the effects of polymers and SLS concentrations on response parameters. Based on the preliminary studies, the quantities of polymers were determined. The concentration of the three polymers (HPMC, HPC, Methocel E5) and SLS quantity were selected as independent variables. At the same time, the three dissolution parameters, MDT, times 25 and 90 % of the drug released ($T_{25\%}$ and $T_{90\%}$), were identified as responses since a single response optimization is thought to yield misleading results (122).

The in vitro dissolution parameters and APX release profiles of the sixteen formulations (APX 1 - 16) are presented in Figure 4.4 and Table 4.8. APX release was affected by the polymer type and amount used in each formula. The cumulative percent released after 24 hrs of each of the APX formulations was complete except for APX 10, that only 80 % of the APX were released. The results illustrated that there is a relationship between the concentration of each polymer and its dissolution profile. Formulation with HPMC concentrations between (39.6 - 46.2 %) APX 1, APX 3, APX 10, APX 11, APX 12, and APX 14 showed the lowest cumulative amount of APX release within 20 h. (91.5 %, 89.7 %, 68.4 %, 84.0 %, 75.8 %, and 87.2 %, respectively). When the HPMC concentrations were between 32.8 – 33.7 % (APX 2, APX 7, APX 8, APX 9, and APX 15), a complete release was achieved after 20 hrs of dissolution. While formulas with HPMC concentrations of 26.4 %

showed complete release after 16 hrs, that obviously revealed a strong dependence of % released (selected as a response) on the concentration of polymers. These results are consistent with what was found in other studies, that increasing the concentration of polymer increases the viscosity of the gel layer and retard the drug release (123,124). Add that polymer with a more hydrophobic methoxy group, HPMC, is less likely to form hydrogen bonding with water. Furthermore, within and between the polymer particles, less hydration of the core occurs, and slower drug release is achieved compared to Methocel® (119,125), which explains what was observed when comparing results of APX 1 and APX 3, (where APX 1 has more methocel® than APX 3), a slight increase in the amount of methocel increased the percent of drug released. SLS is added to the formula as a wetting agent for the inherent hydrophobicity of APX (51). As the added quantity of API is 5 mg, the sink conditions were achieved and the SLS quantity showed no effect on the drug release.

Among all the developed formulations, APX 11 which contains HPMC: Methocel E5: HPC in the ratio 46.2 : 10.4: 36.3 gave ER for 24 hr. was selected as the optimum formula.

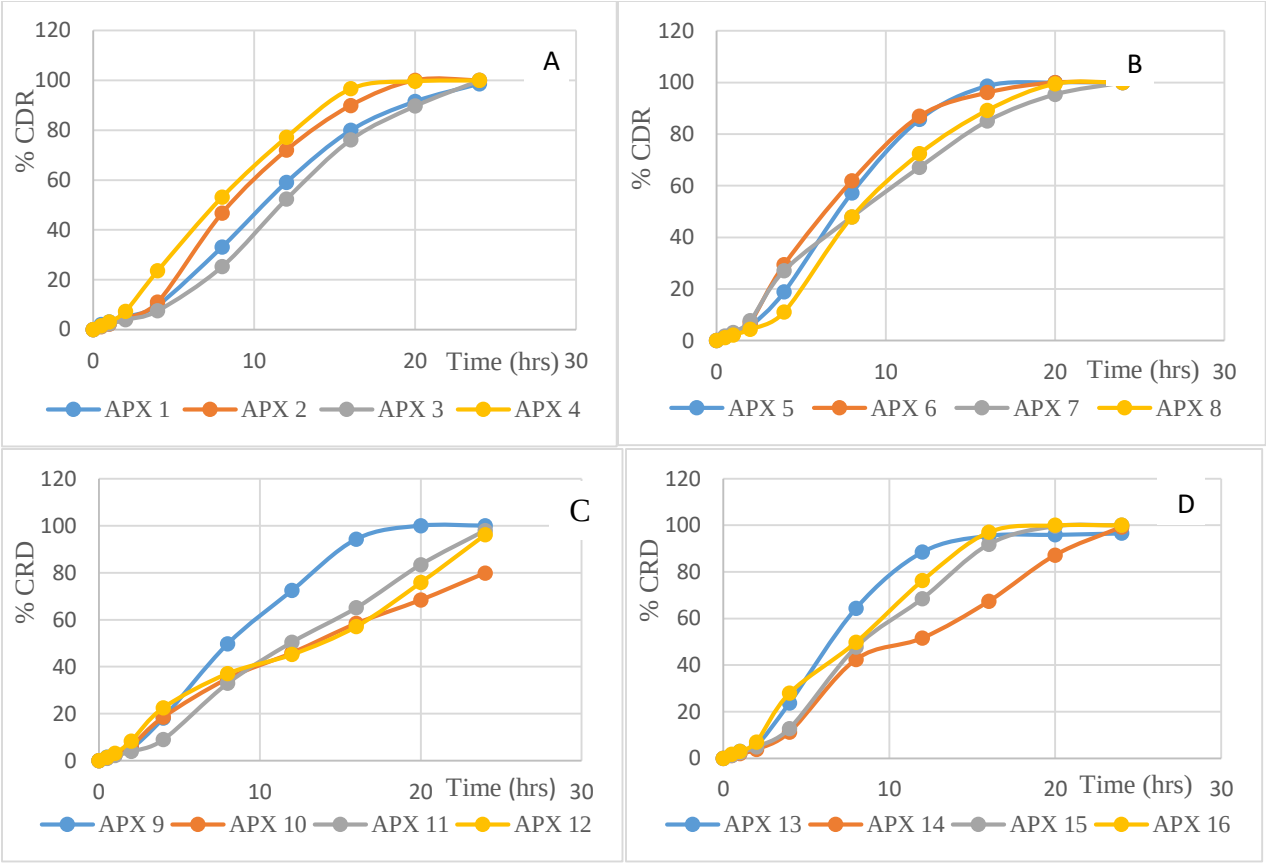

Figure 4.4: In vitro dissolution of APX. formulations; (A) APX 1- 4, (B) APX 5- 8, (C) APX 9-12, (D) APX. 13-16

Table 4.8: In vitro dissolution response parameters:

Formula Code	$T_{25\%}$ (h)	$T_{50\%}$ (h)	$T_{90\%}$ (h)	MDT
APX 1	5.618	11.237	20.227	10.901
APX 2	5.6	8.94	16	9.239
APX 3	6.785	12.293	20.498	12.047
APX 4	4.125	8.135	14.472	8.351
APX 5	3.849	7.699	13.858	7.956
APX 6	3.345	7.153	13.594	7.378
APX 7	3.373	8.945	18.730	9.629
APX 8	4.716	9.399	16.869	9.647
APX 9	4.201	8.851	16.557	8.765
APX 10	5.4	13.25	More than 24	10.756
APX 11	6.103	12.207	21.972	12.177
APX 12	5.951	12.519	23.521	12.177
APX 13	3.778	7.555	13.600	6.886
APX 14	5.886	11.772	21.190	11.567
APX 15	4.680	9.361	16.894	9.599
APX 16	4.050	8.099	14.579	8.318

For CLOP-HS 1, complete drug release was achieved within 15 min, indicating that the used excipients did not retard drug release. The CLOP-HS dissolution profile is illustrated in figure 4.5.

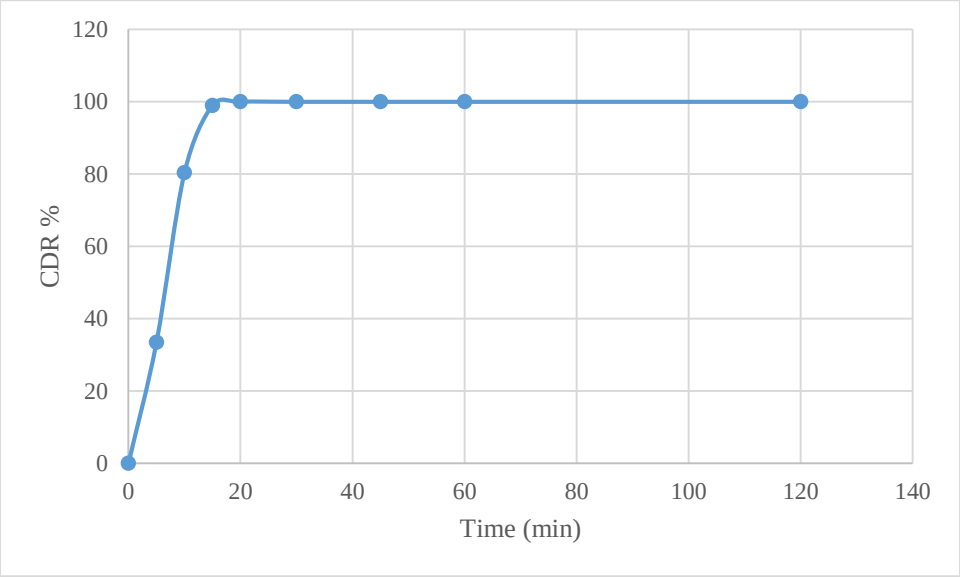

Figure 4.5: In vitro dissolution of CLOP-HS

The dissolution of the final encapsulated FDC after scale up is shown in Figure 4.6. The same dissolution results were obtained, as a complete release of the APX 11 was achieved after 24 hrs, and within 15 min of CLOP-HS 1, with slight rapid release in the percent of CDR of APX 10 mg, two tablets of APX 11, compared to 5 mg, one tablet of APX 11. The total amount of SLS in the dissolution media of 10 mg dose tablets is doubled among the same volume of dissolution media compared to 5 mg. SLS is a surfactant that enhances the solubility of APX, which may be the cause behind the higher % CDR for the dose of 10 mg compared to 5 mg (57).

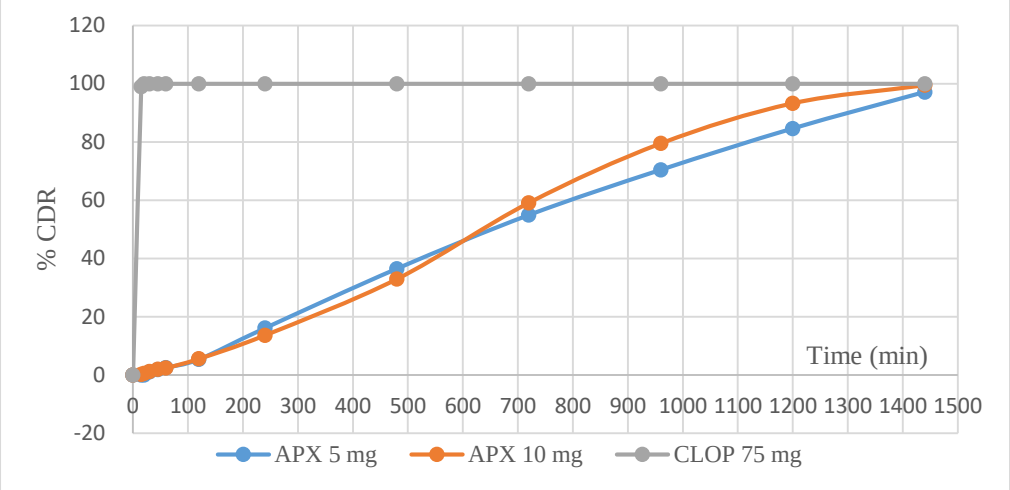

Figure 4.6: In vitro drug release of the final dosage form of two doses of APX.5 mg and APX. 10 mg. (n=6)

4.3.6. Mathematical model analysis:

A mathematical model was constructed to quantify the effect of the variables on the response parameters within the experimental design boundaries. Table 4.9 summarizes the coefficients of model terms. For each response, ANOVA test model results were evaluated and revealed that the sequential p-value was less than 0.05 for each response, and the lack of fit p-values were more than 0.05. The value of R^2 was greater than 0.7, the difference between the predicted R^2 and R^2 was less than 0.2, and the adequate precision values were greater than 8. These values represented a validated design with well fitted responses and insignificant model errors. All responses are shown to be fitted with a linear model.

Table 4.9: ANOVA analysis for the selected different responses of APX formulas.

Response	Model	Sequential p-value	Lack of fit p-value	R^2	Adjusted R^2	Predicted R^2	Adequate Precision	F-value
T25%	Linear	<0.0001	0.712	0.8697	0.8372	0.7412	15.0793	26.71
MDT	Linear	0.0001	0.7175	0.8165	0.7706	0.6501	10.8904	17.79
T90%	Linear	<0.0001	0.9236	0.8377	0.7972	0.6737	13.1650	20.65

Evaluating the effect of each component, a significant association was found between the concentration of polymers (HPMC, Methocel E5, and HPC) and the release time of APX. ($p < 0.001$, $p = 0.0003$, and $p = 0.0006$), respectively. An increase in the HPMC concentration was found to retard APX release while increasing Methocel E5 concentration enhances APX release at the beginning, then slowing the drug release. For HPC, increasing its concentration showed to delay the release of APX at first, then enhancing the release rate lately. SLS different quantities showed to have no significant effect on the release process ($p = 0.4843$). Table 4.10 and figure 4.7 represent the p-values for the 3 evaluated responses.

Table 4.10: Estimated coefficients for responses.

Component	MDT		T25%		T90%	
	p-value	Co-efficient	p-value	Co-efficient	p-value	Co-efficient
HPMC	<0.0001	+0.305009	0.0001	+0.147837	<0.0001	+0.652761
Methocel E5	0.0003	+0.031030	<0.0001	-0.019282	0.0018	+0.08737
HPC	0.0006	+0.024017	0.0066	+0.027684	<0.0001	-0.058459
SLS	0.4843	+1.66680	0.0533	+2.43533	0.5933	+2.59188

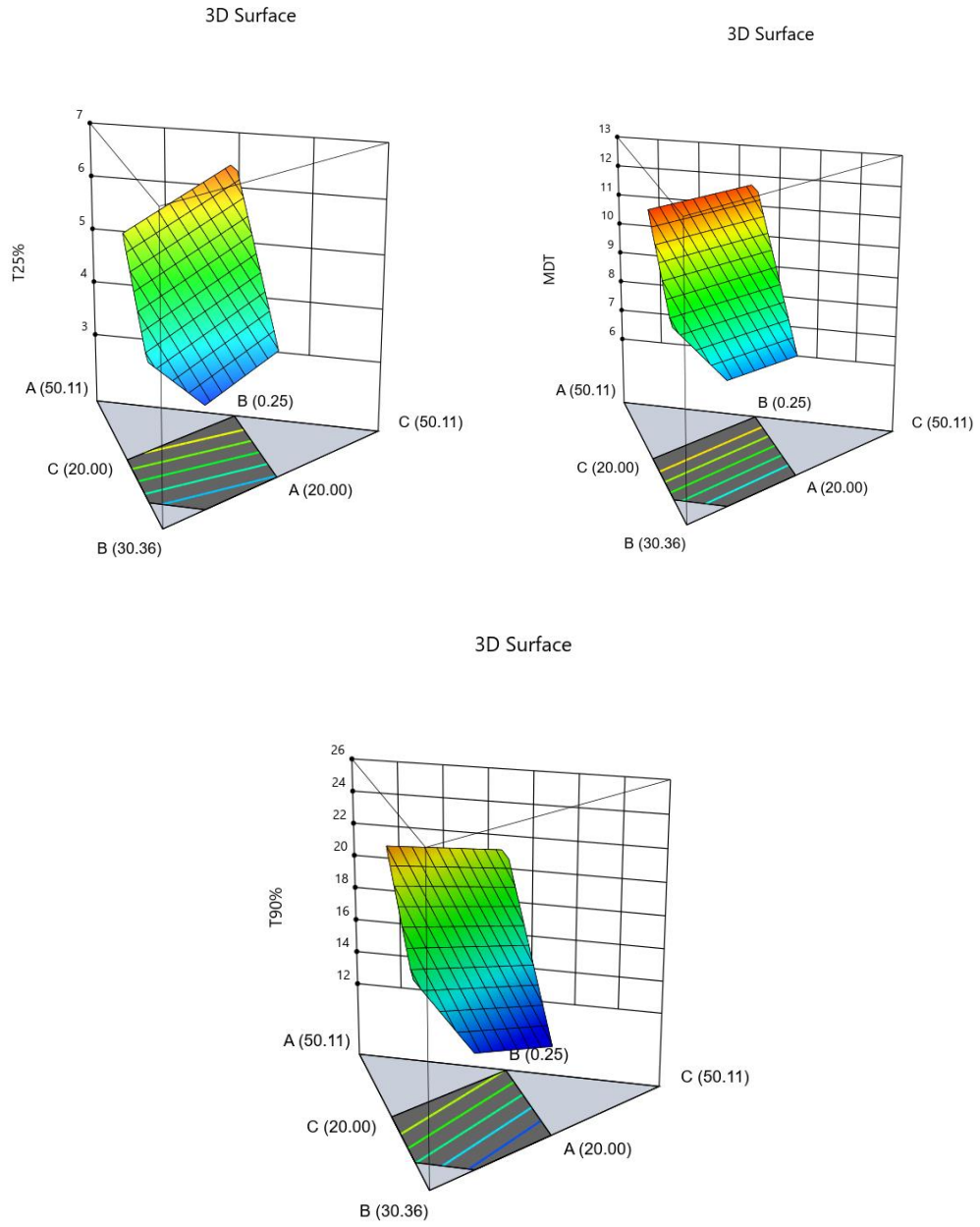

Figure 4.7: 3D response –surface showing the influence of independent variables on responses.

4.3.7. Drug release kinetic study:

In order to determine the drug release kinetics from the prepared sustained release matrix tablets, the dissolution data of the sixteen formulas were fitted into different kinetic models including; zero order, first order, Higuchi, Hixon-Crowell, and Krosmeyer- Peppas models. The regression coefficient (R^2) was close to 1 reflecting the most suitable model for selection (95). Table 4.11 shows the obtained data. Most of the formulated matrices fitted well into zero order, combined with Krosmeyer-Peppas model. As APX has low water solubility and is formulated into a hydrophilic matrix, it is expected to follow zero order kinetics (95). These results indicate that both diffusion of the drug from the swelling gel layer and erosion of this layer are the drug release mechanism. Among formulas having "n" higher than 0.89, the release follows the super case II transport mechanism. Reviewing the behavior of hydrophilic polymers such as HPMC and HPC with low water soluble API revealed that the hydrophilic polymers swell upon hydration, then the drug dissolved and diffused out the system, then the matrix dissolved which explains the erosion process for drug release (126,127). APX formulas 7 and 10 fitted the Hixson-Crowell model, and the "n" value was between $0.45 < n < 0.89$, which indicates the non-Fickian diffusion (anomalous diffusion) model.

Table 4.11: In vitro release kinetics parameters:

Formula code	Zero order		First order		Higuchi Model		Hexon Crowell Model		Korsmeyer- Peppas Model		
	k_0	R^2	K_1	R^2	k_H	R^2	k_{HC}	R^2	k_{KP}	R^2	N
APX 1	4.450	0.9815	0.079	0.9309	17.615`	0.8596	0.022	0.958	4.574	0.9792	0.991
APX 2	5.345	0.9778	0.094	0.9217	19.204	0.8413	0.027	0.9502	5.059	0.9748	1.02
APX 3	4.315	0.9749	0.072	0.8997	16.875	0.8185	0.021	0.931	2.997	0.9814	1.158
APX 4	6.202	0.9854	0.104	0.9419	19.889	0.8512	0.030	0.9918	5.891	0.9935	1.022
APX 5	6.499	0.9794	0.111	0.9173	20.743	0.8265	0.032	0.9468	5.684	0.977	1.054
APX 6	6.672	0.9736	0.122	0.9457	21.632	0.8655	0.035	0.9698	8.295	0.9725	0.915
APX 7	4.835	0.9701	0.099	0.9669	19.549	0.9235	0.027	0.9868	8.779	0.9860	0.794
APX 8	5.337	0.9763	0.094	0.9245	19.204	0.8438	0.027	0.9522	5.258	0.9763	1.005
APX 9	5.508	0.9814	0.101	0.9356	19.940	0.8660	0.029	0.9630	6.276	0.9796	0.952
APX 10	3.518	0.9864	0.056	0.9901	14.148	0.9173	0.016	0.9963	5.511	0.9944	0.845
APX 11	4.098	0.986	0.067	0.9276	16.121	0.8443	0.019	0.9591	3.241	0.9873	1.086
APX 12	3.892	0.9878	0.064	0.9533	15.554	0.8933	0.018	0.9690	4.755	0.9877	0.935
APX 13	6.624	0.9655	0.119	0.9313	21.372	0.8446	0.034	0.9572	7.577	0.9609	0.947
APX 14	4.254	0.9886	0.073	0.9407	16.855	0.8671	0.021	0.9635	4.353	0.9862	0.994
APX 15	5.341	0.9812	0.094	0.9264	19.223	0.8482	0.027	0.9543	5.203	0.9785	1.010
APX 16	6.173	0.9940	0.104	0.9436	19.841	0.8562	0.03	0.9674	5.990	0.9930	1.014

4.3.8. Swelling and erosion studies:

The swelling and erosion plots are shown in figure 4.8, and the swelling tablets and dried residue are illustrated in figure 4.9. The plot revealed that the matrix tablets undergo both swelling and erosion spontaneously. The first two hours showed very rapid water uptake with no erosion, that related to water diffusion into the system, relaxation of the polymer chain, and volume expansion upon exposure to biological fluid (127). Then, water uptake was the dominant process till twelve hours, followed by matrix erosion as the predominates process. After the first 0.5 hrs, an increase in weight was observed even after drying, that may be due to entrapped water within the matrix that cannot evaporate after drying. These results ascertain

that the drug release was ruled out according to zero order, combined with Krosmeyer - Peppas model.

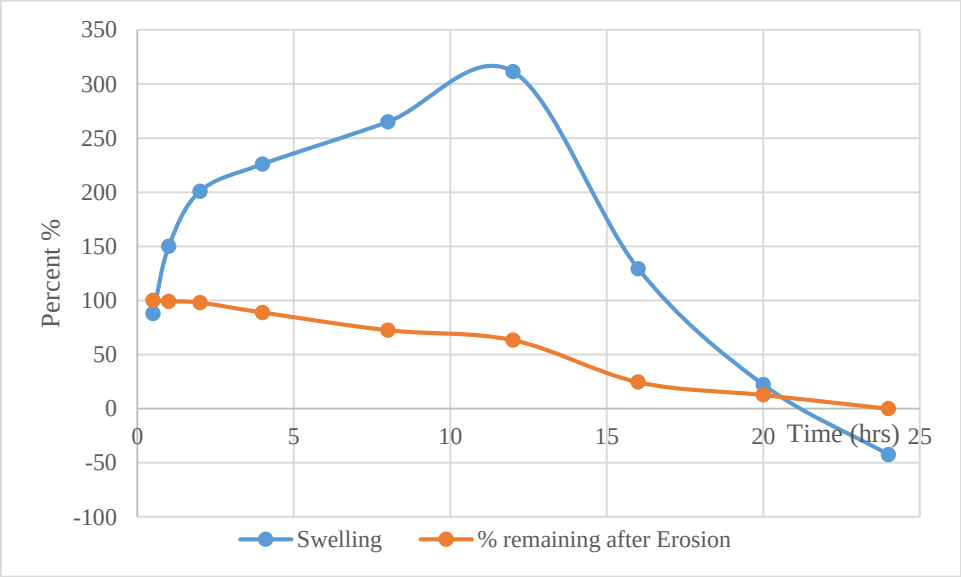

Figure 4.8: swelling and erosion study results

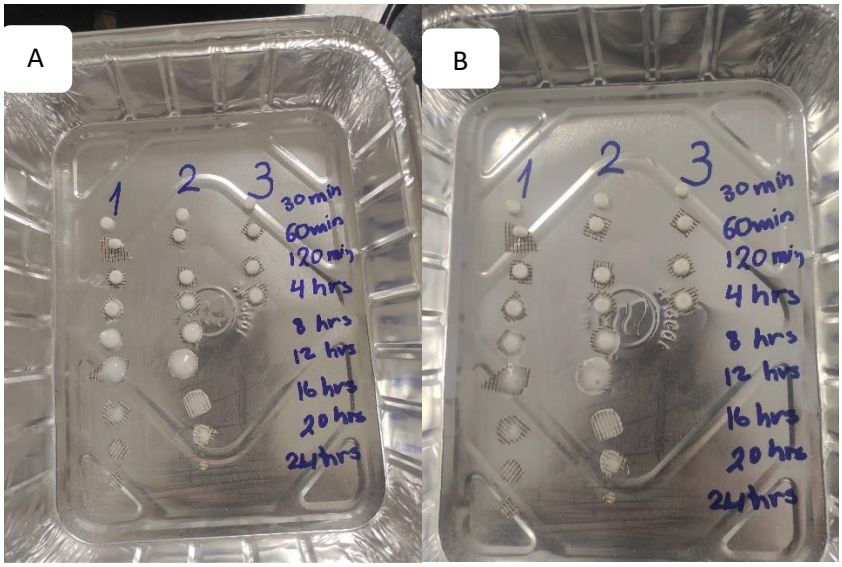

Figure 4.9: Swelling erosion study; A. swellable tablets, B. the remaining tablets after drying for erosion study.

4.3.9. Stability study:

A short term stability study for FDC tablets is shown in table 4.12. The study was carried out on the optimized formulation, a capsule containing (CLOP-HS 1 and APX 11), for three months at 40 ± 2 ^{o}C/ 75 ± 5 % RH. Stability studies have shown no significant changes in the appearance and % of APX drug content. For CLOP-HS, a slight decrease in the active substance assay was found, that related to the presence of sodium stearyl fumarate in the formula, that Sherman found around 1% of COP-HS was degraded after applying stress conditions. He revealed that SSF is a more efficient lubricant than castor oil and PEG, and has a lower degradative effect than magnesium stearate, calcium stearate, zinc stearate, and stearic acid (87). So, it was considered that the formulation has good stability, and a long-term stability study needed to be performed.

Table 4.12: Stability studies of optimized (APX 11) batch

Parameter		**Appearance APX./ CLOP-HS.**	**Drug content APX./ CLOP-HS.**
Initial	APX	Good appearance	95.77%
	CLOP	Good appearance	99.8 %
After 3 months	APX	Good appearance	95.74%
	CLOP	Good appearance	97.55%

Chapter V: Conclusion

5. **Conclusion:**

Oral dosage forms are the most popular route of administration. The FDC tablet offers safety and efficacy advantages by improving patient medication adherence, decreasing polypharmacy, and reducing medication costs. In addition, MPS for FDC products is a valuable tool for preparing medications with different dissolution profiles, pharmacokinetics, and pharmacodynamics. APX and CLOP are antithrombotic substances indicated for patients diagnosed with AF who had ACS and undergone PCI. The formulation of a novel FDC tablet of CLOP and APX will improve patient compliance and facilitate the dosing regimen.

This study developed a novel FDC for CLOP-HS and APX as IR and ER tablets respectively, and evaluated the in vitro dissolution profile of the prepared tablets. The study also developed a simple, rapid, and accurate HPLC analytical method for quantification of the amount of APX and CLOP-HS in the prepared dosage form. The results revealed that an efficient HPLC method was developed, optimized, and validated to separate the anticoagulant APX and antiplatelet CLOP-HS in FDC tablets according to ICH guidelines. In addition, analysis time, resolution, and peaks' quality were optimized and evaluated. The method was 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.

For formulation, CLOP-HS 1 prepared using spray dried lactose as diluent gives a formula with good characteristics and a fast disintegrated effect, and sodium stearyl

fumarate showed good compatibility with CLOP-HS. For ER formula, the type and quantity of used polymers are shown to be important factors that can affect the drug release from the matrix. Full factorial design 2^4, D- optimal level was applied to achieve extended drug release for 24 hrs. Among all the developed formulations, APX 11 which contains HPMC: Methocel E5: HPC in the ratios 46.2: 10.4: 36.3 gave ER for 24 hrs was selected as the best formula. The drug release kinetics follows Korsemeyer-Peppas combined with zero order, and the mechanism was found to be super case II transport. The stability studies indicate that the selected formula was stable.

Further study may be implemented to evaluate the dissolution kinetics of FDC in simulated gastric and intestinal media mimicking fasting and fed conditions, and to perform a bioequivalence study.

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