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Formulation design, development and optimization of Apixaban INN tablet a new era on immediate release drug delivery system

Monoj Kumar Shaha, Md. Asmat Ullah, Md. Mehdi Hasan and Md. Khairul Mamun

Abstract
The oral drug delivery system which includes the solid dosages form such as conventional dosages form and immediate release dosages form. Tablet is most popular among the all dosages forms today and recently found mostly accepted tablet dosages forms. Because of its convenience easy to administration, convenience of self-administration, compactness and easy for the manufacturing. In number of cases immediate onset of action is required than conventional therapy. The basic approach used in development immediate release solid dosages form by using superdisintegrant like sodium starch glycolate (Primogel, Explotab), Polyvinylpyrrolidone (PVP) etc. which provides in instantaneous disintegration of tablet after administration. By using various techniques in can be formulate like wet granulation, direct compression etc. Hence its having A new dosage form allows a manufacturer to extend market exclusivity, while offering its patient population a more convenient dosage form or dosing regimen. Prepared batches were evaluated for all pre-compression parameters and post-compression parameters. This method was found to be linear in a concentration range of 5-30 µg/ml of the drug (r^2= 0.999). The low value of % RSD in the precision study indicates reproducibility of the method. The low value of LOD and LOQ suggests the sensitivity of the method. The results of forced degradation studies indicated that the drug was less stable in thermal and photolytic condition and degraded in acidic, basic, oxidative conditions. On the basis of formulation evaluation, batch was found to be promising formulation suitable for the immediate release of apixaban. Results obtained by validation studies suggested that the developed stability indicating assay method is simple, accurate, specific, sensitive and precise. Thus, this method can be used for routine analysis of apixaban formulation and to check the stability testing. Pharmaceutical products designed for oral delivery and currently available on the prescription and over-the-counter markets are mostly the immediate release type, which are designed for immediate release of drug for rapid absorption.

Keywords: Apixaban, Formulation R&D, Tablets, HPLC, Stability indicating assay method

Introduction
Apixaban (fig. 1) [1] is chemically 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl) phenyl]-4,5,6,7-tetrahydro-1*H*-pyrazolo [3,4-c] pyridine-3-carboxamide. It is a new generation of oral anticoagulant drug that selectively inhibits coagulation factor Xa [1]. It is used in thromboprophylaxis in patients following total knee replacement surgery with a desired efficacy and safety profile [2]. FDA approved apixaban (Eliquis, Bristol-Myers Squibb/Pfizer) on December 28, 2012, for the prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation (AF) [3]. Apixaban is not an official drug in any Pharmacopoeia. Literature survey reveals that only one marketed formulation of apixaban is available which is very costly and cannot afford to poor patients. Therefore, a cost-effective formulation as compared to the marketed formulation is developed. Some methods have been reported for their determination of apixaban by HPLC [4] and hyphenated techniques such as UPLC–MS/MS [5], LCMS [6], GCMS [7], either alone or in combination. This paper presents formulation development of apixaban as well as development and validation of stability indicating assay method by using the RP-HPLC technique.
The common analgesic drug Apixaban shows bad dissolution and tableting behavior due to its hydrophobic structure. Additionally its high cohesivity results in low flowability. Another problem in its manufacturing is its high tendency of sticking to the punches. There are three methods of tablet manufacturing with the choice depending upon the dose and the drug's physical properties, such as, compressibility and flow of the blend. Direct compression is a process by which tablets are compressed directly from mixtures of the drug and excipients,

without any preliminary treatment. A simple formula is considered to be composed of an active ingredient, a diluent and a lubricant.

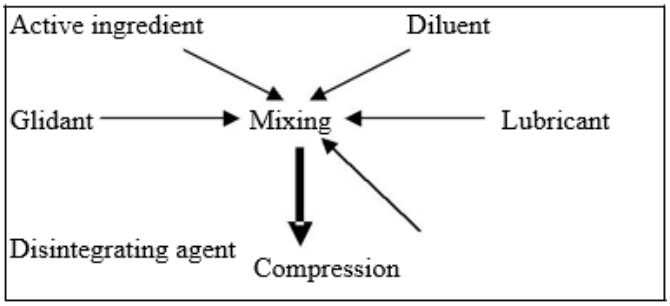


Fig 1: The direct compression process of tablet manufacturing (Armstrong, 2002).

Tablet manufacturing by direct compression has increased steadily over the years. It offers advantages over the other manufacturing processes for tablets, such as wet granulation and provides high efficiency [8]. As direct compression is more economic, reducing the cycle time and straight forward in terms of good manufacturing practice requirements. On the other hand wet granulation not only increases the cycle time, but also has certain limits imposed by thermolability and moisture sensitivity of the active. So pharmaceutical industry is now focusing increasingly on this process [9]. The unnecessary exposure of any drug to moisture and heat can never be justified. Tablets produced by direct compression method give lower microbial levels than those prepared by the wet granulation method. The compaction process exerts lethal effect on the survival of microorganisms. The tablets prepared by direct compression disintegrate into API particles instead of granules that directly come into contact with the dissolution fluid and exhibit a comparatively faster dissolution. The serious limitation of direct compression is the use of more than 30% of the dr.ug in the formulation, mainly for drugs that present low flowability and segregation.

Now days, most of experimentation of tablet formulation development is still performed by changing the levels of each variable (factor) at a time, in an unsystematic way, keeping all other variables constant in order to study the effects of that specific variable on the formulation [10]

The plan of present research is to develop a cost effective Ibuprofen 200mg tablet by direct compression method. The aim is to cut down the time required for disintegration of the tablets in small intestine. Secondarily, direct compression is being studied for its simplicity, cost effectiveness and for its comparatively shorter process. Thus nine different formulations were designed to obtain best optimized product.

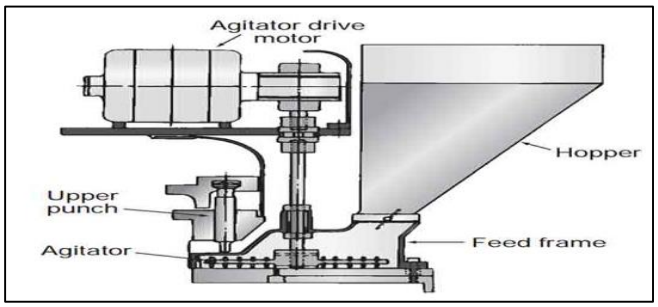
Techniques in direct compression [11]

The processes involved in the manufacture of tablets by direct compression method can be summarized in three steps.

1. Direct compression technique using induced die feeders
2. Direct compression technique using dry binders and
3. Direct compression technique using direct compression excipients

Direct compression technique using induced die feeders

Direct compression technique using induced die feeder is used when formulation ingredients will compact but will not adequately fill the die cavity.



Induced die feeder, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems

Advantages of direct compression technology

The adoption of direct compression technology is based on the following advantages or benefits

1. Direct compression method requires fewer processing steps (unit operations) and less equipment. Therefore, the method is potentially less expensive than other methods used in tablet manufacture.
2. Tablet manufacture can be carried out without the involvement of moisture and heat. Hence, product stability is almost guaranteed.
3. Some direct compressible excipients possess inherent disintegration properties e.g., microcrystalline cellulose.
4. Tablets produced by direct compression method generally show faster dissolution times than those prepared by wet granulation. This is because tablets manufactured by direct compression method disintegrate into primary particle state unlike those manufactured by wet granulation method which breaks down into granules and finally into primary particle state.
5. Changes in dissolution profile are less likely to occur in tablets manufactured by direct compression (if stored for a long time) than in those prepared by wet granulation.
6. Because direct compression excipients have a relatively high binding capacity, the pressure required to manufacture the desired hardness is, in general, less with direct compression vehicles than with conventional granulations, resulting in both higher production rates and longer machine life.
7. Lubrication is performed in the same vessel as powder mixing, thereby reducing both transfer losses and contamination of equipment.

Materials and Methods

Materials

Commercially available tablets of apixaban (5 mg) were procured from local market and apixaban API was obtained from an approved supplier. All excipients were obtained from Active Fine Chemical Ltd., Dhaka, Bangladesh. HPLC grade solvents used in this study were obtained from Active Fine Chemical Ltd., Dhaka, Bangladesh.

Instruments

Apixaban tablets were formulated on single punch tablet compression machine Mini press 1 (Karnavati Engineering limited). The friability test was performed on Electrolab EF-2 friabilator USP. Disintegration test was performed on Electrolab disintegration tester ED-2L. The dissolution testing was performed on Labindia DS 8000. The method was performed on Shimadzu LC 2010 CHT, Japan having a quaternary system with automatic injection facility and UV-Visible detection system. The column used was Purospher

Star RP-18e (5 µm, 250x4, 6 mm), LC solution software and Shimadzu AY-120 balance was used for this work.

Formulation of tablets

Before formulation and pre-formulation studies (organoleptic properties, solubility, and drug excipient compatibility studies) were carried out. Apixaban tablets were formulated by using 32factorial design as presented in table 1. Drug, binder, super-disintegrant and other excipients were weighed separately for 60 tablets per batch as per proposed formulations. The proposed formulations were coded as F1, F2, F3, F4, F5, F6, F7, F8 and F9. The amounts of drug and excipients are expressed in mg (milligram) unit. Initially, the binder, super-disintegrant and other excipients were passed through sieve no. 40. Then, apixaban (API) was added, mixed properly for 5-10 min and sieved again. Blended mass was taken in the hopper and then die and punch were adjusted to get the desired weight of the tablet (100 mg). Tablets were prepared using flat face round 6.5 mm diameter punch by the direct compression process.

Table 1: Batches designed by using 32factorial design

Ingredients	Formulation code (mg/tablet)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Apixaban	5	5	5	5	5	5	5	5	5
Lactose	49	54	44	40	50	45	47	42	52
Microcrystalline Cellulose	30	30	30	30	30	30	30	30	30
Croscarmellose Sodium	3	3	3	7	7	7	5	5	5
Magnesium Stearate	2	2	2	2	2	2	2	2	2
PVPK-30	10	5	15	15	5	10	10	15	5
SLS	1	1	1	1	1	1	1	1	1

Evaluation of tablets

Appearance

Take about 20 tablets in a watch glass and observe visually with white to off white background Check the color, shape and size.

Identification

The chromatogram of the sample preparation exhibits a major peak for Apixaban, the Retention time of which corresponds to that exhibited in the chromatogram of the standard Preparation as obtained in the assay.

Average Weight:

Weigh 20 tablets individually at random basis and record the weight.

$$\text{Average weight} = \frac{\text{Weight of 20 tablets (mg)}}{20}$$

Weight Variation

Find the highest and lowest tablet weight from the average weight measurement. Calculate the positive deviation and negative deviation as follows.

$$\% \text{ of Positive deviation} = \frac{(\text{Highest tablet weight} - \text{Average weight}) \times 100}{\text{Average weight}}$$

$$\% \text{ of Negative deviation} = \frac{(\text{Lowest tablet weight} - \text{Average weight}) \times 100}{\text{Average weight}}$$

Hardness, Thickness & Diameter

Take 10 tablet and measure Hardness (in kp), Thickness (in mm) & Diameter (in mm) with LABINDA test tablet tester. Take average value of Hardness (in kp), Thickness (in mm) & Diameter (in mm) as result.

Friability

Take 20 tablets; remove any loose dust with soft brush. Weigh the tablets (W_1) and place the tablets in the drum of Friability Tester Put the tablet in Friability Tester. Run the instrument at 25 RPM for 4 minutes and remove any loose dust from the tablets as before. If no tablet cracked, split or broken, weigh the tablets (W_2). Calculate the Friability as follows.

$$\% \text{ of Friability} = \frac{(W_1 - W_2) \times 100}{W_1}$$

Disintegration time

Medium : Water

Temperature : 37.0° ± 1.0 °C

Fill the disintegration beaker with sufficient medium and wait to raise the temperature. Take six tablets. Introduce one tablet into each tube and add a disc to the each tube. Suspend the assembly in the beaker containing water and start the operation.

Disintegration is considered to be achieved when no residue, except fragments of tablet, remains on the screen of the test apparatus. Record the disintegration time.

Dissolution

Method: UV Spectrophotometer (UV)

Procedure

Dissolution Medium: Weight 6.9 gm Sodium Dihydrogen Phosphate Monohydrate ($NaH_2PO_4.H_2O$) and 7.1 gm Di-Sodium Hydrogen Phosphate (Na_2HPO_4) with 1000 ml 0.05 % Sodium Lurayl Sulphate. Adjust pH 6.8 with 0.2 M NaOH or 0.2 M H_3PO_4.

Dissolution Condition

Dissolution Medium : 0.05 M Sodium Phosphate Buffer with 0.05% SLS, pH 6.8

Apparatus : USP Type II (Paddle)

Volume : 900 ml

RPM : 75

Time : 45 min's

Vessel Temperature : 37 ± 0.5° C

Standard Solution preparation

Weigh equivalent to 27.75 mg Apixaban working standard to a 100 ml volumetric flask and then make volume up to 100 ml

with Dissolution medium. Shake 20 minutes for sparingly soluble and sonicare for 15 minutes to dissolved. Transfer 1 ml of standard solution to a 100 ml volumetric flask with Dissolution medium.

Sample Solution preparation
Set the dissolution parameters of the instrument as mentioned above. Individually place one tablet in 6 dissolution vessel, 900 ml of dissolution medium which has been equilibrated to the temperature of 37 ± 0.5°c.Immediately start the apparatus and run for 45 minutes. At the end of specified time withdraw 15 ml solutions from zone midway between the surfaces to the dissolution medium and top of the rotating blade not less than 1 cm from the vessel wall. Filter the solution through whatman filter paper into beaker.

Procedure: Determine the amount of Apixaban equivalent dissolved by using UV absorption at wavelength maximum absorbance at 280 nm on filtrate portion of Standard and Sample Solution.
Calculate % of content Apixaban using following formula:

$$\frac{AT}{AS} \times \frac{Ws}{100} \times \frac{1}{100} \times \frac{900}{LC} \times P$$

Where
A_T = Absorbance of Sample preparation of Apixaban
A_s = Absorbance of standard preparation of Apixaban
W_s = Weight of standard preparation of Apixaban
P = Standard potency of Apixaban
LC = Label claim

Assay
Method High Performance Liquid Chromatography (HPLC)

Apparatus
1. Volumetric Flask (1000 ml, 100 ml, 50 ml)
2. Cylinder 100 ml
3. Funnel & whatman Filter Paper
4. Glass Vial (sample holder)
5. Pipette (2 ml, 5 ml, 10 ml)

Reagents
1. Distilled Water
2. Acetonitrile

Procedure
Mobile Phase : Water : Acetonitrile = 50 : 50
Diluent : Mobile Phase

Standard Solution preparation
Accurately weigh and transfer about equivalent to 10 mg of working standard of Apixaban into 100 ml volumetric flask. Add 30 ml of Diluent & sonicare for 10 minutes and volume then up to the mark 100 ml with Diluent and sonicare 2 minutes. Filter this solution with 0.45 µ membrane filter before injection.

Sample Solution preparation:
Ten tablet were weight accurately average weight was calculate and crush the tablet into powder well. Accurately transfer and weight the powder equivalent to 10 mg of Apixaban to a 100 ml volumetric flask. Add 30 ml of Diluent & sonicare for 15 minutes and volume then up to the mark 100 ml with Diluent and sonicare 2 minutes. Filter this solution with 0.45 membrane filter before injection.

Chromatographic condition:
System : Gradient
Column : C_{18} column, (250mm × 4.6mm; 5µm)
Column Temperature : 40 ^{0}C
Flow rate : 1.0 ml / min.
Detection : Spectrophotometer at 280 nm
Injection volume : 20 µl
Run time : 7.0 minutes
Retention time : 3.5 minutes (approximately)

System Suitability
Separately inject the equal volume of standard solution of six replicate injection into the equilibrate HPLC system & calculate RSD. The RSD for six replicate injections should not be more than 2.0%. Then inject Sample solution & record the chromatogram.

Procedure: separately injects equal volumes (about 20 µL) of the standard preparation and the assay preparation of sample solution into the chromatograph, record the chromatograms, and measure the area responses for the major peaks.
Calculate % of content Apixaban using following formula

$$\frac{AT}{AS} \times \frac{Ws}{Wu} \times \frac{Ps}{100} \times \frac{Av.wt}{LC} \times 100$$

Where
A_T = Area of Sample Preparation of Apixaban
A_s = Area of Standard Preparation of Apixaban
W_s = Weight of Standard Preparation of Apixaban
W_u = Weight of sample of Apixaban
P_s = Standard potency of Apixaban
LC = Label claim

***In vitro* dissolution study**
Dissolution studies of each batch were conducted according to USP apparatus II paddle method with 900 ml phosphate buffer (pH 6.8) at 37 °C and 75 rpm. After 5, 10, 20, 30, 45 and 60 min interval samples (10 ml each) were withdrawn from the dissolution medium and replaced with fresh medium to maintain a constant volume. The samples were filtered through a 0.45µ membrane filter. Then samples were diluted to a suitable concentration with phosphate buffer (pH 6.8). Then, the solution was injected into HPLC system. The cumulative percentage of drug release was calculated [12-15].

Assay of tablets
Twenty tablets were weighed and average weight was calculated. These tablets were crushed and powdered in a glass mortar. The tablet powder equivalent to the average weight of apixaban was accurately weighed, transferred to a 50 ml of volumetric flask and diluted up to mark with water: acetonitrile (60:40v/v). The solution was filtered through Whatman filter paper no.. This solution was further diluted to obtain 30 µg/ml with diluent and the sample solution was injected into HPLC system. This procedure was repeated in triplicate.

Forced degradation studies
To evaluate stability, apixaban was subjected to force degradation under the condition of acid, base, neutral hydrolysis and oxidation as per international conference on harmonization (ICH) guidelines [16-19].

Acid hydrolysis
100 mg of apixaban was weighed accurately and transferred to 100 ml volumetric flask containing 100 ml of 0.1N hydrochloric acid (HCl). This mixture was refluxed at 80 °C. After 2 h, 5 ml of refluxed sample was withdrawn and neutralized with 5 ml of 0.1 N sodium hydroxide. This solution was further diluted 10 times with mobile phase to obtain a concentration of 100 µg/ml. The chromatogram obtained after 2 h of acid hydrolysis is shown in fig. 6(a).

Alkaline hydrolysis
100 mg of apixaban was weighed accurately and transferred to 100 ml volumetric flask containing 100 ml of 0.1N sodium hydroxide (NaOH). This mixture was refluxed at 80 °C. After 2 h, 5 ml of refluxed sample was withdrawn and neutralized with 5 ml of 0.1 N hydrochloric acid. This solution was further diluted 10 times with mobile phase to obtain a concentration of 100 µg/ml. The chromatogram obtained after 2 h of alkali hydrolysis is shown in fig. 6(b).

Oxidative degradation
100 mg of apixaban was weighed accurately and transferred to 100 ml volumetric flask containing 100 ml of 3% hydrogen peroxide (H2O2). This mixture was refluxed at 80 °C. After 2 h, 5 ml of refluxed sample was withdrawn. This solution was further diluted 10 times with mobile phase to obtain a concentration of 100 µg/ml. The chromatogram obtained after 2 h of oxidative degradation.

Thermal degradation
100 mg of apixaban IR tablet powder sample was weighed and transferred into 100 ml volumetric flask. The contents were refluxed as such on a water bath previously maintained at 80° C for 2 h. The sample was allowed to cool to room temperature and then the volume was made up to the mark with mobile phase and mixed well. The solution was filtered through a 0.45µ syringe filter and analyzed. The chromatogram obtained after 2 h of thermal degradation.

Photolytic degradation
Photolytic degradation of the drug was carried out by exposure of about 100 mg of apixaban IR tablet powder sample to UV radiation for 12 h. Then the sample was transferred into 100 ml volumetric flask. The sample was allowed to cool to room temperature and then the volume was made up to the mark with mobile phase and mixed well. The solution was filtered through a 0.45µ syringe filter and analyzed. The chromatogram obtained after 12 h of photolytic degradation.

Validation of the method
The developed chromatographic method was validated for system suitability, linearity, range, accuracy, precision, LOD-LOQ and robustness parameters as per ICH guidelines [20-23].

Linearity and range
Working standard solutions were injected in the range of 5-30 µg/ml under the optimized chromatographic conditions and peak areas were calculated at 280 nm. The calibration curve was plotted between areas against concentrations of the drug. Linear regression data, as well as calibration curve.

Precision
Repeatability study was carried out with six replicates and intermediate precision studies were carried out with three concentrations of apixaban with three replicates. The values of % relative standard deviation (% RSD) of precision study are shown in table

Accuracy
The accuracy of the method was determined by calculating percent recovery of the drug by standard addition method. Percent recovery of apixaban was determined at three different level 80%, 100%, and 120% of the target concentration in triplicate. The results of accuracy study are shown in table

Robustness
Robustness of the optimized method was studied by changing flow rate (±0.1 ml/min), change in wavelength (±1 nm) and change in mobile phase composition (±5%) during analysis. The sample was injected in triplicate for every condition and % RSD was calculated for each condition is shown in table

Limit of detection (LOD) and limit of quantitation (LOQ)
Five sets of concentrations were prepared between 5-30 µg/ml and the corresponding areas of these sets were measured. Calibration curves were plotted for each set. The standard deviation of the y-intercept and average slope of the calibration curve was used to calculate LOD and LOQ using following formulae. LOD=3.3×SDS LOQ=10×SDS
Where SD is the standard deviation of y-intercepts of the calibration curves; S is the mean slope of six calibration curves.

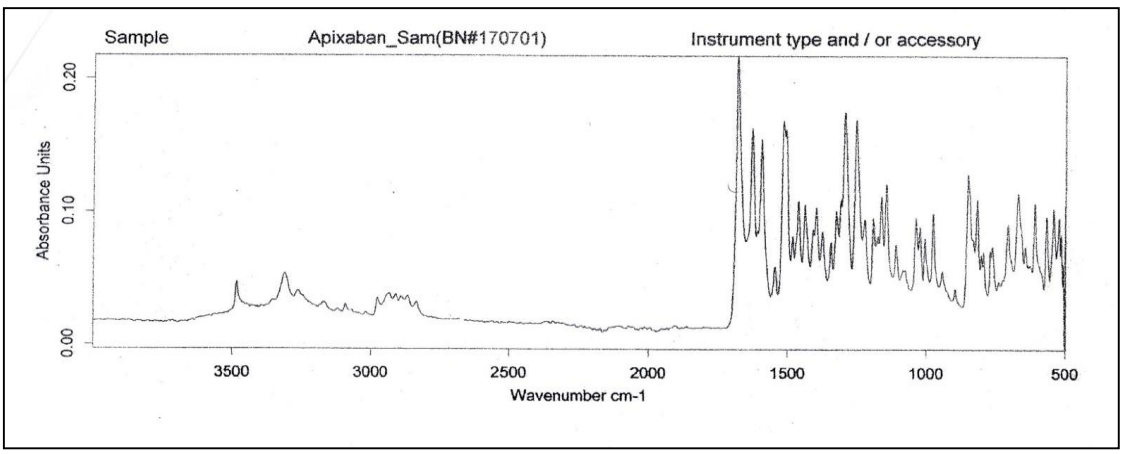


Fig 2: IR spectrum of apixaban

Results and Discussion

Formulation of tablets

Apixaban was found to be soluble in acetonitrile. Drug-excipients interaction studies were performed using FTIR spectrophotometer.

The FTIR spectra for the formulation and pure drug are shown in fig. 2 and fig. 3. Characteristics peaks obtained for the pure drug correlated well with that of the formulation peaks. This indicated that the drug was compatible with the formulation components.

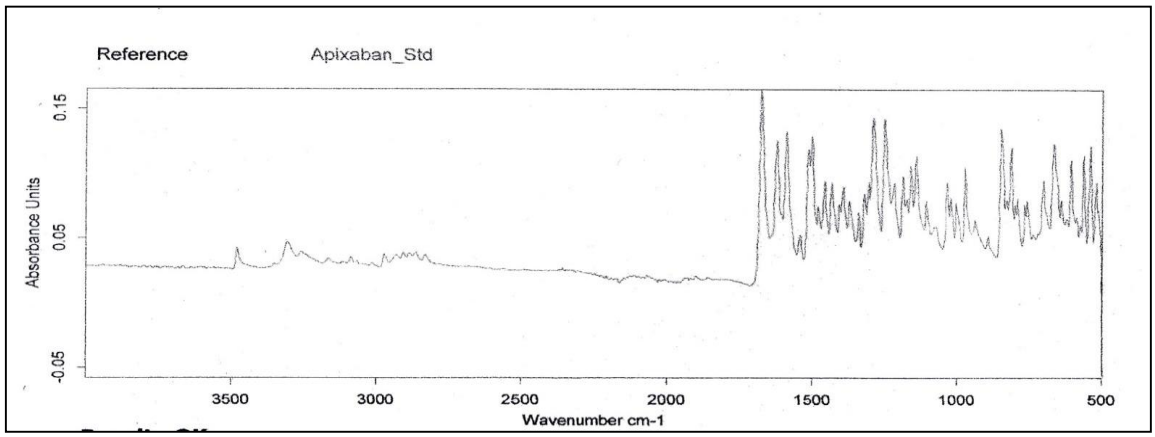


Fig 3: IR spectrum of apixaban formulation

Evaluation of powder blend

The angle of repose of all formulations was between 21.37 ° to 28.14 °, while the result of the Carr's index and Hausner ratio was between 11.71% to 20.02% and 1.03 to 1.25, respectively.

Evaluation of powder blend characteristics is presented in table 3. The results indicate that the prepared powder mixtures have acceptable flow properties and compressibility. All pre-compression parameters are found to be within the acceptance criteria.

Table 2: Evaluation of powder blend characteristics

Parameters	F1	F2	F3	F4	F5	F6	F7	F8	F9
Angle of Repose	23.05 °	22.89 °	23.49 °	21.37 °	25.55 °	25.01 °	27.07 °	27.09 °	28.14 °
Bulk Density	0.606	0.571	0.588	0.606	0.588	0.588	0.588	0.571	0.588
Tapped Density	0.689	0.714	0.666	0.714	0.689	0.714	0.666	0.714	0.689
Hausner's Ratio	1.13	1.25	1.13	1.17	1.17	1.21	1.03	1.25	1.17
% Carr's Index	12.04	12.02	11.71	15.12	14.65	17.64	17.97	20.02	14.65

(n=3)

Evaluation of tablets

The evaluation parameters of all formulation batches are presented in table 3. The thickness of tablets was observed in the range of 2.4±0.172 to 2.5±0.171 mm, it was found that all prepared tablets had a uniform thickness. The hardness of tablets was in the range of 3.4±0.05 to 3.7±0.10 kg/cm2, which shows sufficient mechanical strength. The total weight loss of the prepared tablets due to friability was found in the range of 0.50% to 0.80%, which is within acceptance criteria. The weight variation of prepared tablets was in the range of 98.43±0.61 to 100.82±0.79. The content uniformity of the prepared tablets was in the range of 93.98±1.32% to 95.25±1.12%, which reveals content uniformity. All evaluation parameters were found within the acceptance criteria.

Table 3: Evaluation of formulation batches of tablets

Parameters	F1	F2	F3	F4	F5	F6	F7	F8	F9
Thickness (mm)*	2.5±0.18	2.4±0.17	2.5±0.17	2.5±0.17	2.4±0.17	2.4±0.17	2.4±0.17	2.5±0.17	2.5±0.17
Weight	98.43±0.6	99.06±0.7	99.08±0.7	99.41±0.9	100.11±0.8	99.49±0.8	100.6±0.8	100.82±0.7	100.48±0.6
Variation (mg)*	1	8	2	3	4	3	1	9	7
Hardness kg/cm2*	3.4±0.05	3.6±0.05	3.5±0.15	3.5±0.05	3.6±0.20	3.5±0.05	3.7±0.10	3.4±0.05	3.6±0.20
Friability (%)	0.50	0.60	0.80	0.60	0.80	0.60	0.60	0.60	0.50
Disintegration test	138±1.15	137±1.80	136±2.11	137±1.45	136±1.23	139±2.10	140±2.40	145±1.76	139±1.32
Content	94.62±0.5	94.62±1.8	94.62±1.6	94.30±0.7	93.98±1.32	94.62±1.8	95.25±1.1	94.93±0.43	95.25±1.85
uniformity	4	5	8	2		7	2		
In vitro	36.48	40.25	29.97	38.95	90.979	69.25	62.55	44.59	67.57
Dissolution									
Studies									

(*n=3) data represented as mean±SD

it is observed that formulation batch F5 shows maximum drug release pattern as compared to other formulations. Therefore, this batch was selected as optimized formulation batch.

Optimization of chromatographic conditions

UV spectrum of apixaban showed maximum absorbance was found at 280 nm. Hence, 280 nm was selected for detection

wavelength of this drug. Initially, various chromatographic conditions were tried in order to obtain better separation characteristics by changing mobile phase composition and pH. Finally, the mobile phase containing water: acetonitrile (60:40 v/v)+0.1 % TEA was selected at 1 ml/min flow rate. The retention time of apixaban was found to be 5.66 min. The chromatogram of apixaban is shown in fig. 5 and optimized chromatographic conditions are mentioned in table 6.

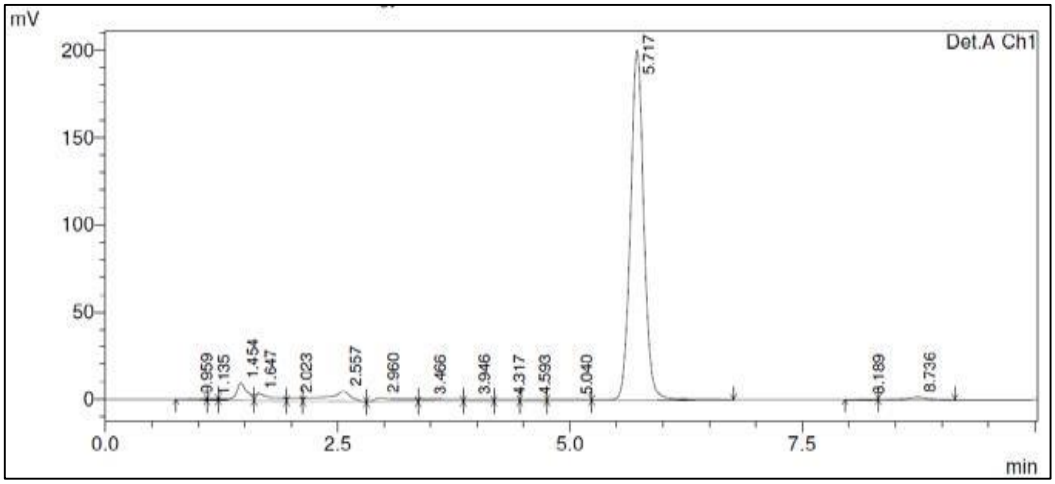


Fig 4: UV spectrum of apixaban

Assay of tablet formulation

The drug content was calculated as an average of three determinations and assay results were shown in table 7. The results were very close to the labeled value of commercial tablets. The value of mean % drug was found to be 99.31% which is within acceptance criteria.

Forced degradation studies

Chromatograms obtained under different stress conditions like acidic, alkaline hydrolysis, oxidative, thermal and photolytic degradation are presented in fig 6(a), 6(b).

Table 6: Optimized chromatographic conditions

Parameters	Details
Mobile phase	Water: Acetonitrile (60:40) v/v+0.1 % TEA
Column	Purospher Star RP-18 end-capped (5 µm) Hibar 250x4, 6
Flow rate	1 ml/min
Detection	280 nm
Injection volume	20 µl
Run time	8 min
Retention time	5.66 min
Diluent	water: acetonitrile (60+40) v/v+0.1 % TEA

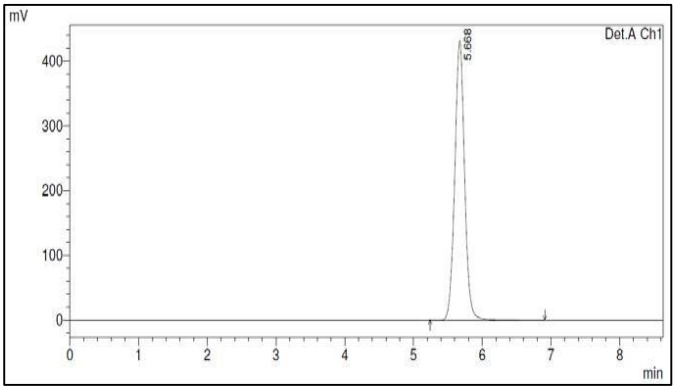


Fig 5: Chromatogram of apixaban

Table 7: Results of assay of apixaban

S. No.	Sample solution concentration (µg/ml)	Actual concentration found	Amount of drug estimated mean±SD*
1	30	29.82	
2	30	29.74	99.31±0.16
3	30	29.81	

*The value is represented as a mean±SD of 3 observations.

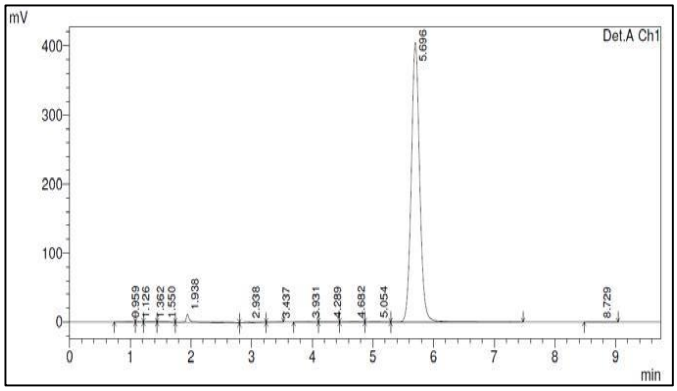


Fig 6(a)

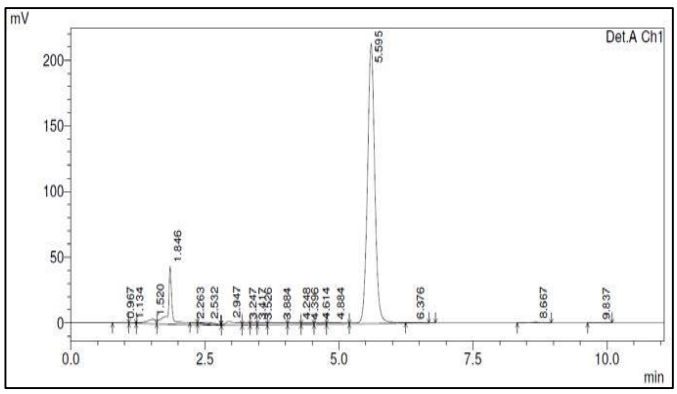


Fig 6(B)

The first chromatogram obtained by acid hydrolysis [fig. 6(a)] suggested that 9.15% degradation of the drug was found, when refluxed at 80 °C for 2 h in 0.1 N HCl. The major degradation product formed was at 1.938 min retention time. This study indicates that apixaban was susceptible to acid hydrolysis. Second chromatogram obtained by alkaline hydrolysis [fig. 6(b)] indicated that apixaban was not stable to alkaline hydrolysis when refluxed at 80 °C for 2 h in 0.1 N NaOH. The Value of % degradation was found to be 14.86% and major degradation products appeared at 1.84 and 2.94 min retention time. The third chromatogram obtained by oxidative suggested that 4.15% degradation was observed when refluxed with 3% H2 O2 at 80 °C for 2 h. The major degradation products appeared at 1.45, 1.64 and 2.55 min retention time. Fourth chromatogram obtained by thermal degradation indicated that apixaban is less degraded when refluxed at 80 °C for 2 h. The minor degradation product was obtained at 1.45 min retention time. Fifth chromatogram obtained by photolytic degradation indicated that apixaban is less degraded upon exposure to UV radiations for 12 h. The minor degradation product was obtained at 1.45 min retention time.

Precision

The repeatability, intra-day precision was calculated as the relative standard deviation of results from three samples, during the same day, and the inter-day precision was studied by comparing on two different days. The percent relative standard deviation (% RSD) was calculated which is within the acceptable criteria of not more than 2 were shown in table 8.

Table 8: Repeatability and intermediate precision for apixaban

Precision	Concentration of drug (µg/ml)	Mean area±SD*	% RSD
Repeatability*	20	799 859±1636.10	0.37
	10	377 252±6808.76	1.80
Intra-d	20	835 741±3499.53	0.42
	30	121 438±2744.61	0.23
Inter-d	10	373 366±3209.56	0.86
	20	801 115±8040.09	0.10
	30	117 499±1936.27	0.16

*Each value is represented as a mean±SD of n observations. The value of n is 6 for repeatability study and 3 for intraday and interday precision. SD: Standard deviation, %RSD: Percent relative standard deviation

Development of polynomial equation

From the data of in vitro drug release of factorial formulation A_1 to A_9 polynomial equation for in-vitro drug release was derived using design expert 8.0 software. The polynomial equation for 3^2 factorial designs is:

$$Y= b_0+ b_1X_1+ b_2 X_2 + b_{12} X_1X_2 +b_{11}X_1^2+ b_{22}X_2^2$$

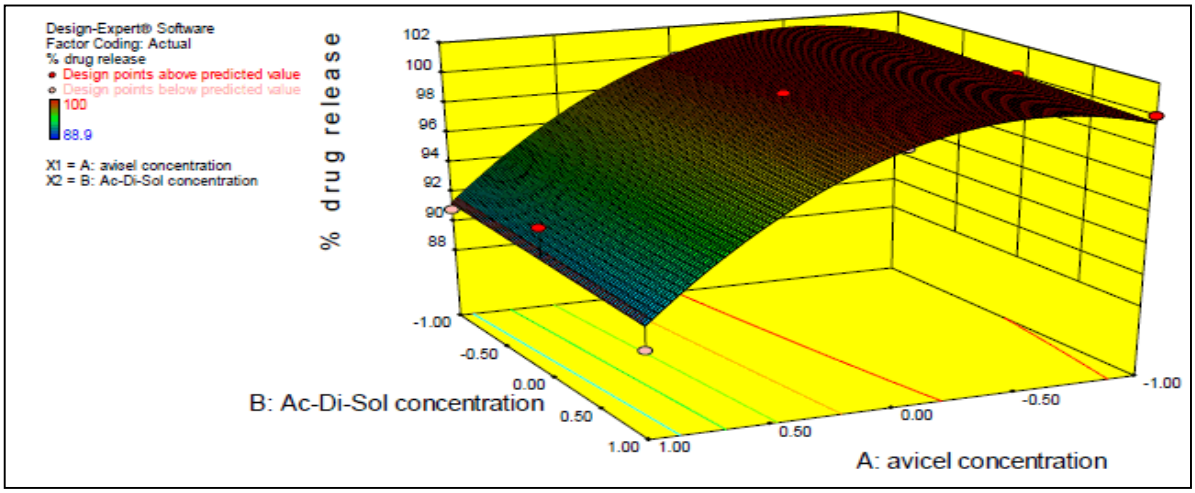


Fig 8: 3D response –surface showing effect of factorial variables on % drug release.

Accuracy

The accuracy was determined by the standard addition method. Amounts of 6; 10; 14 µg/ml of the apixaban standard were added to the sample solution in which 10.0 µg/ml of the drug had been incorporated previously. The final concentrations of the fortified solutions were 16.0, 20.0 and 24.0 µg/ml of apixaban. The recovery experiments were performed in triplicate for each concentration. The value of mean % recovery and % RSD (table 9) at each level was found within acceptance criteria that indicate the method is accurate.

Table 9: Accuracy of apixaban

Levels	Amount taken (µg/ml)	Amount found (µg/ml)	% recovery	% Mean recovery± % RSD
80%		16.36	102.2	101.25±0.17
	16	15.72	98.25	
		16.54	103.3	
100%		19.36	96.8	96.1±0.63
	20	19.15	95.75	
		19.15	95.75	
120%		27.88	92.93	93.03±0.13
	24	27.95	93.16	
		27.90	93.00	

*Percent recovery was done in triplicate, % recovery: Percent recovery, %RSD: Percent relative standard deviation

Robustness

Robustness was performed by changing various method parameters like a change in flow rate, the composition of mobile phase and change in detection wavelength. Finally, the effect of these changes was not deliberate. The value of % RSD (table 10) was found to be within acceptance criteria which showed the reliability of the method.

Table 10: Robustness study of apixaban

Parameters	% RSD
A: Change in flow rate	1.98%
0.9 ml/min	
1 ml/min	
1.1 ml/min	
B: Change in Mobile Phase	1.64%
Water: ACN (55:45) v/v+0.1% TEA	
Water: ACN (60:40) v/v+0.1% TEA	
Water: ACN (65:35) v/v+0.1% TEA	
C: Change in wavelength	1.25%
279 nm	
280 nm	
281 nm	

*Each value is represented as % RSD of n observations. The value of n is 3 for change in flow rate, change in wavelength and change in mobile phase composition. %RSD: Percent relative standard deviation

Limit of detection (LOD) and limit of quantitation (LOQ)

The sensitivity of measurement of apixaban by use of proposed methods was estimated in terms of the limit of quantitation (LOQ) and limit of detection (LOD). The values of LOD and LOQ have been found to be 1.020µg/ml and 3.091µg/ml, respectively. These values show that method is sensitive.

Conclusion

Direct compression is becoming one of the most common and economical method of tablet manufacturing in the pharmaceutical industry. Although the principles governing direct compression have been well known for many years, the technique has only recently become more established as a result of the introduction of certain grades of excipients specifically designed for direct compression. A cost-effective formulation of apixaban tablets was developed by 32 factorial design. Results of formulation evaluation of prepared between indicate that batch F5 is a promising formulation for the immediate release of the drug. Results obtained by validation studies suggested that the developed stability indicating assay method is simple, accurate, specific and precise. Thus, this method can be used for routine analysis of apixaban formulation and to check the stability testing.

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