World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086

Available online at: http://www.wjpsonline.org/
Original Article

Investigation on Polymorphs of Apixaban, an Anticoagulant Drug: Study of Phase Transformations and Designing Efficient Process for their Preparation

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Received: 25-01-2015 / Revised: 03-03-2015 / Accepted: 05-03-2015

ABSTRACT

The present work describes the investigation on the polymorphs of Apixaban (**1**), an anticoagulant drug obtained by following basic synthetic process known in the prior art. Polymorphs present inherently in the basic process are identified, synthesized and characterized. Robust and efficient process for the preparation of apixaban (**1**) and their different polymorphs has been described based on the understanding of solvent mediated phase transformations, solid-solid phase transformation and suitable medium in which phases are effectively formed. Process optimization details conducted towards the synthesis of identified polymorphs of apixaban with high chemical and polymorphic purity are presented.

Keywords: Apixaban, Atrial fibrillation; Coagulation factor Xa inhibitors Anticoagulant, Polymorph transformation

INTRODUCTION

The term polymorph has been derived from a Greek word "poly", which means "many", and "morph" means "form" hence polymorphism is the ability of a substance to exist in two or more crystalline phases that have different arrangements of the molecules in the crystal lattice.[1]Active pharmaceutical ingredients (APIs) delivered to the patient in the solid-state dosage form can exist in varied solid forms as polymorphs, pseudo-polymorphs, salts, co-crystals and amorphous solids. More than 50% of active pharmaceutical ingredients (APIs) are estimated to have more than one polymorphic form.[2]These various solid forms often display different mechanical, thermal, physical and chemical properties that can extraordinarily influence the bioavailability, hygroscopicity, stability and other characteristics of the drug. Hence, a thorough understanding of the relationship between the particular solid form of an active pharmaceutical ingredient (API) and its functional properties is important in selecting the most suitable form of the API for development into a drug product.[3]Polymorphism is a natural property of the solids and are not created or invented as they are merely discovered as a part of routine experimentation. They are the result of the experimental conditions under which the API is obtained. Any compound that presents polymorphism will naturally tend to its more stable form even without any human intervention. Polymorphism can affect the shelf life, solubility, formulation properties, and processing properties of a drug.[4]A particular polymorph of a drug can be more effective than another, easier or more difficult to manufacture, or even dangerous.[5]

Polymorph provides patenting opportunities to the inventor to patent new polymorphs of the known molecule which intern gives an advantage to extend period of market exclusivity.[6] In order to be patentable, a polymorph has also to be "inventive" and an inventive step is a technical problem solved by the invention. Thus, polymorphism has now become a scientifically challenging issue for the pharmaceutical companies and legally challenging even for the courts.[7]Independent patent applications on polymorphs have become increasingly frequent and controversial since they can be used to delay the entry of the competitors. Thus the polymorphs can be considered with in the prior art and therefore

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non patentable if they are inevitably obtainable by following the basic patent process for the active pharmaceutical ingredient. It is very difficult for the court to determine the novelty of the polymorph in absence of report that describes the inherent existence of claimed polymorphs. Once the polymorphs are identified, an efficient process for making them at production level and stabilizing them for a longer period to maintain the integrity is a challenging task to a process chemist involved in the development of active pharmaceutical ingredients in the pharmaceutical industries. Herewith we present a case study where in polymorphs inherently present in the basic synthetic process are identified and characterized. Their stability and inter conversion pattern is thoroughly understood before establishing the production friendly process for making each one of them.

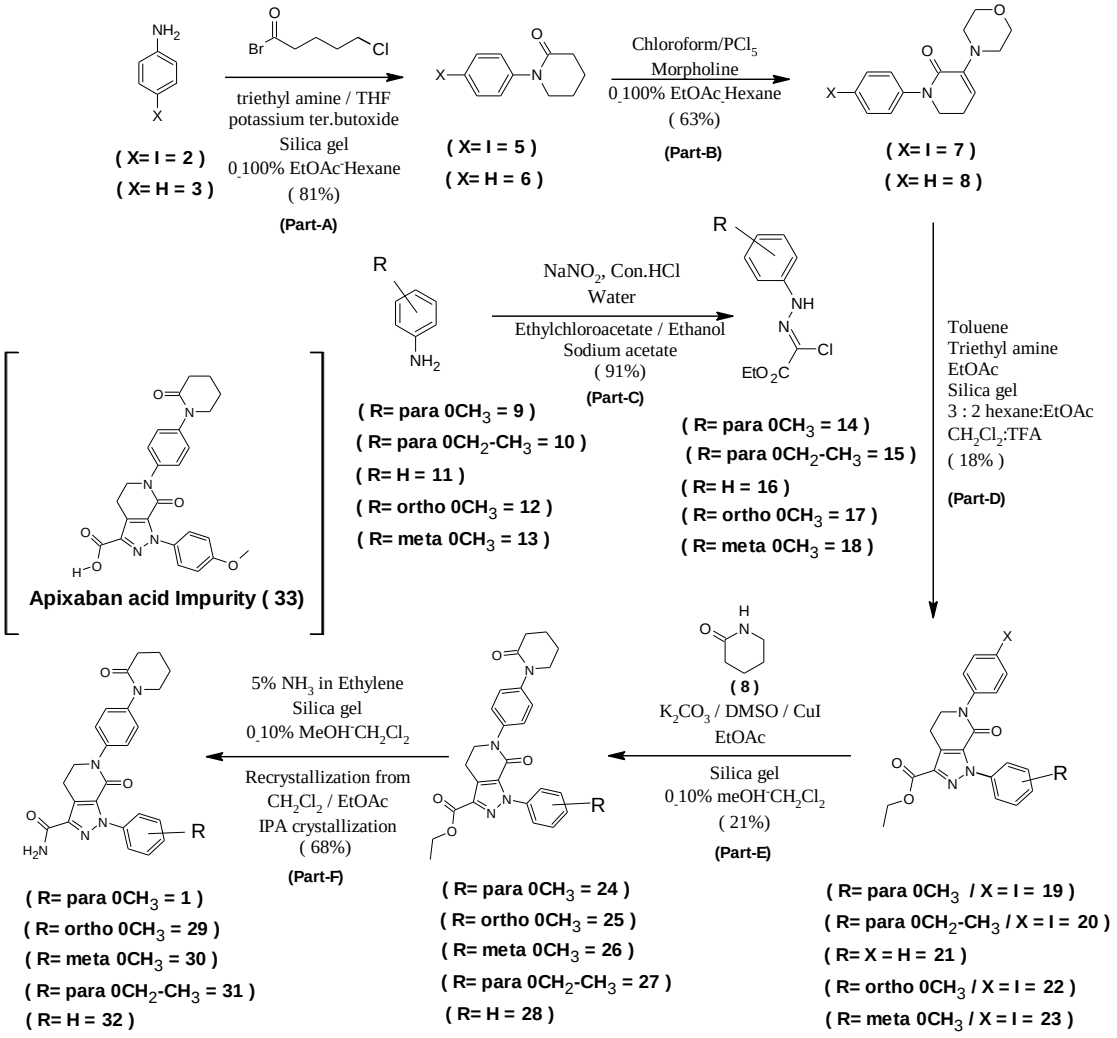
Apixaban is an anticoagulant drug chemically known as l-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-l-yl) phenyl]-4, 5, 6, 7-tetrahydro-lH-pyrazolo[3,4-c]pyridine-3-carboxamide and sold under the brand name Eliquis to treat the people with atrial fibrillation (a heart rhythm disorder) to lower the risk of stroke caused by a blood clot. It was invented by Aderis pharmaceuticals and was developed jointly by Pfizer and Bristol-Myers Squibb. Apixabanis a selective, reversible, direct inhibitor of factor Xa indicated to reduce the risk of stroke and systemic embolism in patients with non-valvular atrial fibrillation.[8]Eliquiswas approved both in US and Europe on December 2012 and January 2010 respectively. Eliquis is also used after hip or knee replacement surgery to prevent a type of blood clot called deep vein thrombosis (DVT), which can lead to blood clots in the lungs (pulmonary embolism).

The first synthetic method[9]reported for apixaban **(1)** involved condensation of two key intermediates **7** and **14** in toluene in presence of triethylamine to obtain morpholine intermediate which was purified by column chromatography using 3:2 ethyl acetate-hexane and further reacted with trifluoro acetic acid in dichloromethane to obtain iodo compound **19** as a foam in 18% yield. The iodo compound **19** was condensed with *δ*-valerolactam (**8**) in DMSO in presence of copper iodide and potassium carbonate to obtain crude ester compound (**24**) which was further purified by column chromatography using 0-10% methanol-chloroform as eluent to furnish pure ester **24** as a tan foam in 21% yield. Finally the ester compound **24** was reacted with 5% ammonia under pressure in ethylene glycol in a sealed vessel to obtain crude apixaban (**1**) which was precipitated by adding water. Purification of crude apixaban was done by silica gel chromatography using 0-10% methanol-dichloromethane as eluent to afford apixaban (**1**) as white solid. Further a portion of the obtained solid was recrystallized from mixture of dichloromethane-ethyl acetate to afford apixaban (**1**). The remaining solid obtained by column chromatography purification and material recovered from filtrate of recrystallization was further recrystallized from isopropyl alcohol to afford additional apixaban (**1**) with 68% yield (Scheme 1).No description of a crystal form/polymorph has been mentioned at different isolations/purifications in the above reported process. Thus it is very difficult to understand whether the polymorphs reported subsequent to the basic process are really novel or they fall within the scope of first reported synthetic method. Originator[10] reported form N-1 (Anhydrous form) and form H2-2 (dihydrate form) and several other researchers[11] have reported many other polymorphs for **1.** This made us to explore the polymorphs of apixaban (**1**) which are inherently associated with the reported synthetic method.

According to the originator' subsequent report[10a]form N-1has characteristic peaks at 10.0, 10.6, 12.3, 12.9, 18.5 and 27.1±0.2° 2θ; and form H2-2has characteristic peaks at 5.8, 7.4, 16.0, 20.2, 23.5, and 25.2and 27.1±0.2°2θ. Further, Vladiskovic et al.,[11g]reported another polymorph namely form-α (sesquihydrate form) which has characteristic peaks at 6.0, 7.1, 11.0, 11.9, 12.9, 13.6, 15.1, 16.1, 17.6, 19.1, 20.3, 21.6, 22.7, 24.5, 26.0, 26.7, 27.2, 28.8, and 30.1±0.2° 2θ.

We thus investigated the details of the polymorphs obtained at each step. With the exploration of first reported synthetic method in our hands the obtained results are summarized below. Aminolysis of ester **24** with 5% ammonia in ethylene glycol was performed in sealed tube. The suspension obtained after completion of the reaction was treated with water to get the apixaban as a precipitate which was filtered and dried under vacuum at 60-65 °C. Wet and dry samples analyzed by PXRD showed that both exhibit form N-1of apixaban. The apixaban obtained above was chromatographed over silica using 0-10% methanol-dichloromethane. Concentration of eluted fractions provided form-α of apixaban. A portion of the solid obtained by column chromatography was recrystallized using dichloromethane-ethyl acetate which again provided form-α of apixaban. Finally crystallization of the remaining solid obtained after column chromatography and the filtrate material from dichloromethane-ethyl acetate re-crystallization using isopropyl alcohol provided form N-1.Further there is a procedure reported[11h]for the conversion of form H2-2 to form N-1 which is cumbersome and requires several

vessels. It also required a transient tank, a receiver tank, monitoring of critical temperature of the transient tank as well as the receiver tank, repeated heating and cooling cycles at varied RMP of reactor. Moreover, it requires in-process checks to ensure the complete transformation of form H2-2 to form N-1. Thus, exploration of the reported synthetic method revealed the information that form N-1 and Form-α inherently exists in the innovator process.[9]



Scheme 1. Reported synthetic approach for Apixaban

Further it was also noticed that purity of apixaban obtained after following the above synthetic method was around 90-93% by HPLC. Chromatographic purification employed to achieve the chemical purity is not feasible at industrial scale and leads to the generation of huge amount of effluents moreover upon crystallization of apixaban using mixture of dichloromethane-ethyl acetate provided apixaban having high levels of dichloromethane as a residual solvent which could not be controlled up to the acceptable limits as per the ICH guideline even after re-drying at higher temperature. Thus the first reported process for preparing apixaban was found to be in efficient to deliver high chemical purity and polymorphic purity and hence we identified four objectives, first to identify the polymorphs which are inherently formed by following first reported process. Second objective was to develop an improved, efficient and production friendly process for the preparation of highly pure apixaban. Third to establish an efficient and commercially viable process for the preparation of each of these polymorphs which are obtained by following the first reported process and the fourth objective was to develop the process to achieve the transformation of these polymorphs.

MATERIALS AND METHODS

Melting points were determined on Analab melting point apparatus, in open capillary tubes and are uncorrected. The ^{1}H NMR (400 MHz) spectra were recorded on a Varian Gemini 400 MHz FT NMR spectrometer. Chemical shifts were reported in parts per million using tetramethylsilane as internal standard and are given in δ units. The solvents for NMR spectra were deuterochloroform and deuterodimethyl sulfoxide unless otherwise stated. Infrared spectra were taken on Perkin Elmer Spectrum 100 in potassium bromide pallets unless otherwise stated. High-resolution mass spectra were obtained with a Shimadzu GC-MS QP mass spectrometer with an ionization potential of 70 eV. All reactions were monitored by High performance liquid chromatography (HPLC) on Agilent Technologies 1200 series. Gas chromatography on Agilent Technologies 7683B with head space was used for analyzing the residual solvents. Samples generated as described in the solid form were typically analyzed by X-Ray Powder Diffraction (XRPD). XRPD was conducted on a Bruker D8 Advance X-ray powder Diffractometer using Cu Kα radiation at 1.54. The instrument was equipped with a fine focus X-ray tube. The voltage and amperage of X-ray generator were set at 40 kV and 30 mA, respectively. The divergence slices were set at 0.3°. The diffracted radiation was detected by a Lynx Eye Detector. Typically, a theta-two theta continuous scan at 4.95°/min (0.4 sec/0.033°step) from 2°2θ to 50° 2θ was used. A Corundum probe standard was used to check the peak position. In general, positions of XRPD peaks are expected to individually vary on a measurement-by-measurement basis by about ±0.2°2θ. Differential Scanning Calorimetric (DSC) analysis was performed with Mettler Toledo DSC823e, USA. The equipment was calibrated with indium. The samples were scanned at 10°C/min from 50-300°C. Thermo Gravimetric Analysis (TGA) was performed on TGA/DSC-1 Weight calibration of TGA was performed by using 50 mg standard reference material. The instrument was programmed to heat the specimen from 25 to 300°C with a heating rate 20°C per min. Common reagent grade chemicals used were either commercially available and were used without further purification or prepared by standard literature procedures.

Synthesis of 1-(4-methoxyphenyl)-6-(4-iodophenyl)-7a-morpholin-4-yl-7-oxo-3a,4,5,6,7, 7a-hexahydro-1*H*-pyrazolo [3,4-c]pyridine-3carboxylic acid ethyl ester (19a). Ethyl acetate (8.0L) and ethyl (2Z)-chloro[(4-methoxyphenyl) hydrazono]acetate (**14**, 1.0 kg) were charged into reactor and stirred at 25-30 °C to obtain clear solution. To the obtained clear solution was charged 1-(4-iodophenyl)-3-morpholin-4-yl-5,6-dihydropyridin-2(1H)-one (**7**, 1.49 kg) and triethylamine (0.788 kg). Contents were refluxed (76-78 °C) till completion of reaction (9-10 h) by HPLC. Reaction mass was cooled to 45-50 °C and ethyl acetate was distilled under vacuum. Water (5.0 L) and dichloromethane (12.0 L X 1, 6.0 L X 1) was added to the residue and stirred for 30 min. Dichloromethane layer was separated, combined, washed with water (5.0 L) and concentrated under vacuum to obtain the crude **19a.** Ethyl acetate (1.5 L) was added to the crude **19a** and distilled under vacuum below 55 °C to obtain the residue again. Ethyl acetate (4.70 L) and methyl tertiary butyl ether (14.10 L) was added to residue and the mixture was heated to reflux temperature (55-60 °C). The suspension was then gradually cooled initially to 25-30 °C and then to 0-5 °C and stirred for 60 min. The precipitate obtained was filtered, washed with methyl tertiary butyl ether (1.0 L) and dried under vacuum at 50-55 °C for 4-5 h to get 1.80 kg (76.6% yield) of **19a** as a crystalline solid. HPLC purity: 99.40%,; 1H NMR (CDCl3): δ 1.36-1.40 (t, 3H), 2.03-2.09 (m, 1H), 2.34-2.38 (bd,1H), 2.47-2.52 (m, 2H), 2.63 (bs, 2H), 3.48-3.51 (m, 2H), 3.70-3.82 (m, 7H), 3.99-4.00 (d, 1H), 4.31-4.37 (m, 2H), 6.50-6.54 (m, 2H), 6.80-6.84 (m, 2H), 7.52-7.56 (m, 2H),7.63-7.67 (m, 1H); MS (ESI, m/z): 605 $[M + H]^+$.

Synthesis of 1-(4-methoxyphenyl)-6-(4-iodophenyl)-7-oxo-4,5,6,-7-tetrahydro-1*H*-Pyraz--olo-[3,4-c]pyridine-3-carboxylicacid ethyl ester (19). Acetic acid (1.0L) and 1-(4-methoxy-phenyl)-6-(4-iodo-phenyl)-7a-morpholin-4-yl-7-oxo-3a,4,5,6,7,7a-hexahydro-1H-pyrazolo[3,4-c]pyridine-3carboxylic acid ethyl ester (**19a**, 1.0 kg), were charged into reactor and stirred for 5-10 min. Water (1.0 L) was then charged to the suspension and heated to 75-80°C for 2-3 h till completion of reaction by HPLC. Reaction mass was cooled to 0-5°C and stirred for 60 min. The precipitate was filtered and washed with water (1.0 L). Wet product was then slurried with 5% w/v solution of sodium bicarbonate (4.0 L) and stirred for 1 h, filtered, washed with water (1.0 L), and dried under vacuum at 50-55 °C for 8-10 h to obtain 0.83 kg of **19** (96.84% yield); HPLC purity: 99.63%; FT-IR (KBr): 3406.57, 3070.41, 2978.54, 2960.58, 2932.35,2900.21, 2835.77, 1712.69, 1677.17, 1607.57, 1591.28, 1557.68, 1541.95, 1487.85, 1416.08,1438.33, 1373.78, 1323.29, 1298.57, 1251.15, 1137.98, 1031.69, 845.66, 830cm^{-1}; ^{1}H NMR (CDCl3): δ 1.40-1.43 (t, 3H), 3.29-3.32 (t, 2H), 3.79 (s,3H), 4.06-4.10 (t, 2H), 4.42-4.47 (q, 2H,), 6.88-6.92 (m, 2H), 7.04-7.08 (m, 2H), 7.42-7.46 (m, 2H), 7.64-7.68 (m, 2H); MS (ESI, m/z): 518 $[M + H]^+$.

Synthesis of 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxo-piperidin-1-yl)- phenyl]-4,5,6,7-tetra hydro-1*H*-pyrazolo[3,4-c]pyridine-3-carboxylic acid ethyl ester (24).[12]Cesium carbonate (1.35 kg, having moisture content 7%) was dehydrate twice in toluene (3.0L X 2 times) to remove the moisture and the anhydrous cesium carbonate obtained was degassed at 50-55°C for 45 min. Residue was then cooled to 25-30°C and vacuum was released using nitrogen gas. To the obtained anhydrous cesium carbonate was charged toluene (22.0L), 1-(4-methoxy-phenyl)-6-(4-iodo-phenyl)-7-oxo-4,5,6,-7-tetrahydro-1H-Pyraz-olo-[3,4-c]pyridine-3-carboxylic acid ethyl ester (**19**, 1.0 kg) and deltavalerolactam (**8**, 0.288 kg) and stirred for 5-10 min under nitrogen atmosphere. Copper iodide (0.115 kg), and *N, N'*-dimethylethylenediamine (0.120 kg) were added to the reaction mixture under nitrogen atmosphere and the mixture was refluxed (106-110 °C) for 15-20 h. Upon completion of reaction by HPLC, reaction mass was cooled to 45-50 °C and toluene was distilled under vacuum to obtained residue. Residue was cooled to 25-30 °C, added dichloromethane (9.0 L) and stirred for 30-45 min at 25-30°C. Reaction mixture was filtered over celite bed to remove inorganic contents and contents were washed with dichloromethane (3.0 L). Combined DCM layer was washed with 15% aqueous ammonia (2.25 L) followed by 31% w/v sodium thiosulfate solution (2.25 L X 2), water (2.25 L) and brine (2.25 L) solution. Dichloromethane layer was then concentrated under vacuum at temperature below 40°C to obtain residue. Ethyl acetate (1.0 L) was added to the obtained residue and distilled out completely below 55 °C to remove the traces of DCM. To the obtained residue was added ethyl acetate (4.0 L), and mixture was heated to 70-75°C, stirred for 45-60 min and the suspension was gradually cooled to 0-5°C. The precipitate obtained was filtered, washed with ethyl acetate (0.5 L X 2) and dried under vacuum at 50-55 °C for 5-6 h to obtain 0.78 kg (82.6% yield)**24** as an off white crystalline solid. HPLC purity: 97.32%; FT-IR (KBr): 3608, 3453, 3042, 2989, 2942, 2839, 1728, 1650, 1674, 1635, 1514, 1459, 1439, 1312, 1252,1146, 1108, 1004, 984, 833, 800, 7587, 609cm-1;^{1}H NMR (CDCl3): δ 1.40-1.43 (t, 3H), 1.90-1.93 (m, 4H), 2.53-2.55 (t,2H), 3.28-3.32 (t, 2H), 3.56-3.59 (t, 2H), 3.79 (s,3H), 4.09-4.13 (t, 2H), 4.42-4.47 (q, 2H), 6.87-6.91 (m, 2H), 7.22-7.23 (m, 2H), 7.30-7.34 (m, 2H), 7.44-7.48 (m, 2H), 7.53-7.58 (m, 1H); MS (ESI, m/z): 489 [M + H]. $^+$

Synthesis of DMF solvate of Apixaban. Ethylene glycol (2.0L) and **24,** (0.250 kg) were charged to autoclave and the contents were heated to 60-65°C for 4-5 h under ammonia pressure (5.0 kg). Upon completion of reaction by HPLC, reaction mass was cooled to 25-30°C and diluted with water (2.0 L). Obtained suspension was extracted with dichloromethane (10.0 L X 1 and 1.5 L X 1). The DCM layer was then washed with water (5.0 L) decolorized with activated carbon (10gm). The dichloromethane layer was concentrated under vacuum to obtain residue (0.23kg).To the obtained residue was added DMF (1.15 L) and the mixture was heated to 80-85 °C for 40-45 min. Solution was then gradually cooled to 0-5 °C. The precipitate obtained was stirred for 1.0 h, filtered, washed with ethanol (0.12 L), and dried under vacuum at 50-55 °C for 6-7 h to obtain DMF solvate of **1**(Yield. 0.212 kg) which was further crystallized in DMF (1.075 L) to remove an unknown impurity observed at the level of 0.27% at RRT 1.08. Yield of pure DMF solvate of **1** was 0.185 kg; HPLC purity: 99.80%, content of **33**: ND; content of **29**: ND; content of **19a**: ND; content of**30**: ND; content of **24**: ND; content of **19**: ND; content of **31**: 0.03%; Single maximum unknown impurity at RRT 1.08: 0.09%; DMF content: 96585 ppm.

Synthesis of Apixaban form N-1. To the DMF solvate (0.190 kg) was added ethanol (1.90 L), acetonitrile (57 ml) and water (513 ml). The mixture was heated to reflux (80-85 °C) for 30 min to obtain the clear solution. Clear solution was cooled to 40-45°C and solution was concentrated under vacuum to remove almost all organic solvents leaving behind the aqueous portion (around 0.6 L). The concentrated mass was further cooled to 25-30°C and added water (1.9 L). Suspension was stirred for 1-2 h. Solid precipitate was filtered, washed with water (0.250 L), and dried under vacuum at 50-55 °C for 6-7 h to provide form N-1 of Apixaban (**1)**. Yield: 0.160 kg. HPLC purity: 99.83%, content of **33**: ND; content of **29**: ND; content of **19a**: ND; content of **30**: ND; content of **24**: ND; content of **19a**: ND; content of **19**: ND; content of **31**:0.03%; Single maximum unknown impurity at RRT 1.08: 0.06%; DMF content: ND. FT-IR (KBr): 3484, 3312, 3262, 3171, 3120, 3091, 3017, 2937, 2833,1670, 1596, , 1682, 1630, 1596, 1505, 1438,1398, 1163,1144, 1038, 1023, 975, 848, 814, 756, 668cm-1; 1H NMR (CDCl3): δ 1.79-1.89 (m, 4H), 2.36-2.39 (t, 2H), 3.18-3.21 (t,2H), 3.57-3.60 (t, 2H), 3.79 (s, 3H), 4.03-4.06 (t, 2H), 6.97-7.01 (m, 2H), 7.26-7.28 (d, 2H), 7.33-7.35 (d, 2H); 7.44 (bs, 1H), 7.48-7.51 (m, 2H), 7.73 (bs, 1H), MS (ESI, m/z): 460 [M + H].

Synthesis of Apixaban form H2-2 using form N-1 or form-α. Acetone (2.0L), apixaban form N-1(0.250 kg), dichloromethane (1.0 L) and water (0.250 L) were charged to the reactor at 25-30°C.

Obtained suspension was stirred for 5-6 h at 25-30 °C. Suspension was then gradually cooled to 0-5°C, stirred for 1.0-2.0 h and filtered, washed with acetone (0.125 L).Obtained wet product was charged to the reactor containing water (3.75 L) and stirred for 1.0-2.0 h. The suspension was filtered, washed with water (0.250 L) and dried under vacuum at 50-55 °C for 8-10 h to provide form H2-2 of **1.** Yield: 0.235 kg; HPLC purity: 99.87%, content of **33**:ND; content of **29**: ND; content of **19a**: ND; content of **30**: ND; content of **24**: ND; content of **19**: ND; content of **31**: 0.03%; Single maximum unknown impurity at RRT 1.08: 0.03%; FT-IR (KBr): 3449, 3299, 3179, 2942, 1670, 1617 , 1514, 1463, 1335, 1299, 1255, 1167,1147, 1031, 1020, 1004,833, 758, 703, 607 cm-1.

Synthesis of Apixaban form H2-2 using apixaban-DMF solvate. Acetone (2.0L), apixaban DMF solvate (0.250 kg), dichloromethane (1.0 L) and water (0.250 L) were charged to the reactor at 25-30°C. Obtained suspension was stirred for 5-6 h at 25-30°C. Suspension was then gradually cooled to 0-5°C, stirred for 1.0-2.0 h and filtered, washed with acetone (0.125 L).Obtained wet product was charged to the reactor containing water (3.75 L) and stirred for 1.0-2.0 h, suspension was then filtered, washed with water (0.250 L) and dried under vacuum at 50-55 °C for 8-10 h to provide form H2-2 of **1.** Yield: 0.201 kg; HPLC purity: 99.85%, content of **33**: ND; content of **29**: ND; content of **19a**: ND; content of **30**: ND; content of **24**: ND; content of **19**: ND; content of **31**: 0.03%; Single maximum unknown impurity at RRT 1.08: 0.04%; DMF content: 1350 ppm

Synthesis of Apixaban form-*α* using form H2-2. Acetone (4.0 L) and apixaban form H2-2(0.250 kg) were charged to the reactor and the suspension was heated to reflux (54-57°C) and stirred for 16-18 h. Suspension was then gradually cooled to 0-5°C and stirred for 1.0-2.0 h, filtered, washed with acetone (0.125 L) and dried under vacuum at 50-55 °C for 10-12 h to obtain form-α of **1**. Yield: 0.233 kg; HPLC purity 99.87%, content of **33**:ND, content of **29**: ND; content of **19a**: ND; content of **30**:ND; content of **24**: ND; content of **33**: ND; content of **19**: ND; content of **31**:0.03%; Single maximum unknown impurity at RRT 1.08: 0.01%; FT-IR (KBr): 3451, 3304, 3181, 2955, 1667, 1611 , 1592, 1514,1465, 1350, 1334, 1299, 1255, 1229, 1167, 1145,1008, 1019, 1002, 980,,833, 758, 703, 607 cm-1.

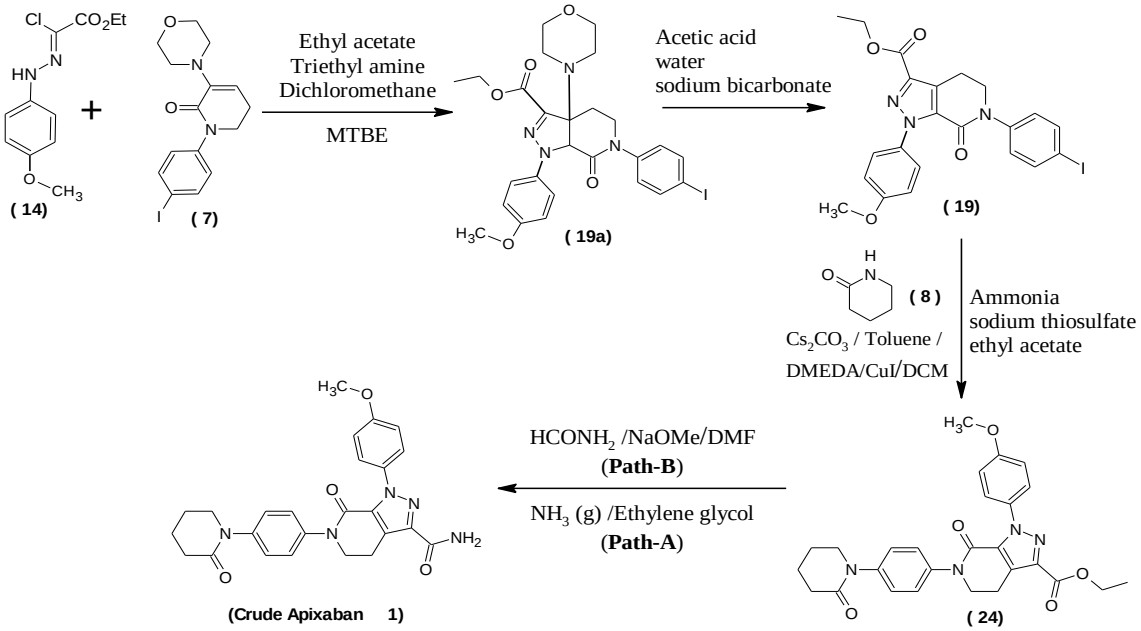
Synthesis of Apixaban form N-1 using form H2-2 or form-α. Isopropyl alcohol (2.5 L) and apixaban form H2-2 or form-α (0.250 kg) were charged into reactor and suspension was heated to reflux at 78-80 °C and stirred for 5-6 h. Suspension was then gradually cooled to 0-5°C and stirred for 1.0 to 2.0 h, filtered and washed with isopropyl alcohol (0.125 L) and dried under vacuum at 50-55 °C for 4-6 h to provide form N-1 of apixaban (**1**). Yield: 0.24 kg; HPLC purity: 99.86%, content of **33**:ND; content of **29**: ND; content of **19a**: ND; content of **30**: ND; content of **24**: ND; content of **31**: 0.03%; content of **19**: ND; single maximum unknown impurity at RRT 1.08: 0.03%;

RESULTS AND DISCUSSION

Study of impurity profile and their control. Overall we have identified six impurities during the development. The impurities **29**, **30**, and **31** were also found to be carried forward as derivatives from the early stage intermediates. The impurities **19** are **24** are the intermediate compounds (Scheme 1). Apart from the above impurities, we noticed the formation of impurity **33** which is process specific and formed only if aminolysis of **24** was carried out by using formamide and sodium methoxide procedure.[11h]Elimination of **33** was very difficult and it could not be easily removed by crystallization process using almost all the known solvents and their mixtures using different process conditions. Hence during our development work we avoided aminolysis of **24** by using formamide and sodium methoxide procedure.

Improved and efficient process for the preparation of highly pure Apixaban (1). Key intermediates **14** was manufactured as per the reported route of synthesis[11h]and **7** was manufactured as per the reported route of synthesis[9]with substantial process improvements to achieve an overall yield of 50%yield over four steps (starting from 4-Iodoanaline) and 99% purity by HPLC.[13]Condensation of **7** and **14** was performed in ethyl acetate in presence of triethylamine as a base at reflux temperature for 9-10 h. Upon completion of reaction by HPLC, ethyl acetate was concentrated under reduced pressure, residue obtained was dissolved in dichloromethane, the solution was washed with water and concentrated under vacuum to furnish the crude morpholine intermediate **19a** which was purified using the mixture of ethyl acetate and methyl tertiary butyl ether to obtain pure morpholine intermediate **19a** with around 76%yield and 99.3% of purity by HPLC (Scheme 2). Obtained morpholine intermediate was then heated in a mixture of acetic acid and water to furnish **19** with yield of 96% (over the reported yield of max 18%) and purity of 99.6%. Substantial yield and quality of **19** were achieved due to isolation and purification of intermediate **19a** and novel process for removal of morpholine using acetic acid and water mixture.

Further, the preparation of **24** from **19** was established by optimizing the Ullman's reaction using different solvents (DMSO, DMF, isopropyl alcohol, 1-4 dioxane, n-butanol, *N*-Methyl-2-pyrrolidone) and different bases (sodium carbonate, potassium carbonate, lithium carbonate, cesium carbonate, tribasic potassium phosphate) in presence of ligands (ethylene glycol, L-proline, trans,1,2-diaminocyclohexane and *N,N'*-dimethyl ethylene diamine) and copper iodide as a catalyst. Among the above combinations, toluene as a solvent, cesium carbonate as a base, *N,N'*-dimethylethylenediamine (DMEDA) as a ligand and copper iodide as a catalyst furnished **24** with appreciable yield of 82% (over reported yield of 21%) and purity of at least 97% by HPLC.As per the optimized process, synthesis of **24** was performed under anhydrous condition and inert atmosphere at reflux temperature for 18-20 h. Completion of the reaction was monitored by HPLC. After completion of the reaction, toluene was distilled out under reduced pressure, the residue obtained was dissolved in dichloromethane and the solution was filtered to remove inorganic contents and finally washed with ammonia solution followed by sodium thiosulfate solution. Concentration of DCM layer under vacuum provided the crude **24**, which was purified from ethyl acetate to obtain pure **24** in 82% yields and around 97% purity by HPLC.



Scheme 2.Improved process for the preparation of apixaban (1): Synthesis of **1** was then explored using an alternative process (Path-A, Scheme 2) as the process according to Path B[10a] required extremely anhydrous condition otherwise this leads to formation of the apixaban acid impurity **(33)** which was very cumbersome to remove by a crystallization process. As per Path A, synthesis of apixaban was performed by heating compound **24** in ethylene glycol under ammonia pressure in an autoclave at 60-65°C for 5-6 hours. After completion of the reaction, the reaction mass was diluted with water and the resulting mass was extracted with DCM, washed DCM layer with water and concentrated under vacuum to yield crude apixaban. To establish the purification process, solubility profile of apixaban in different solvents was studied to identify the solvent for crystallization process (Table 1). Based on the solubility results, we explored purification using DMF, DMSO, acetic acid, *N*-methyl-2-pyrrolidone, and dimethylacetamide wherein apixaban shown considerable solubility. Among them DMF furnished appreciable improvements in the quality of apixaban and thus DMF was chosen as a solvent for optimizing the purification process. Unfortunately, though DMF crystallization furnished desired purity by HPLC but it led to the formation of apixaban as a DMF solvate. To avoid the same we further explored purification using methanol, ethanol, isopropyl alcohol, ethyl acetate, acetone, toluene, acetonitrile, water and their mixtures but were not successful. Thus we established the de-solvating process for apixaban-DMF solvate to achieve the desired polymorph consistently and which is scalable; the details of the same are provided below.

Process for the preparation of apixaban and their polymorphs:

Process for making form N-1: Our main object was to de-solvate the apixaban-DMF solvate using a suitable solvent system to provide apixaban with high chemical purity and desired polymorph simultaneously. It was clear that the compound has to undergo dissolution to de-solvate **1**. However the

solubility study indicated that it had poor solubility in most of the solvents and needs higher volume of solvent for dissolution**.** After screening several solvents and their combination with water, we identified a novel combination comprising mixture of ethanol, acetonitrile and water for dissolution of apixaban in a minimum volume of around 13 to 17 volume per gram of apixaban. A detailed optimization study was conducted to understand the parameters responsible to achieve form N-1 consistently with desired quality. The solvent optimization details are summarized in below table 2.The required volume of solvent mixture for dissolution was observed to be varied based on the chemical purity of apixaban-DMF solvate used for dissolution. It was also noticed that more the chemical purity of apixaban-DMF solvate higher the volumes of solvent mixture (17 volumes max) for dissolution. Thus as per the optimized process, apixaban was suspended in 10v of ethanol and the contents were heated to reflux and to the refluxing mixture was added 3v of mixture of acetonitrile and water having ratio 10:90 respectively to achieve clear solution. In case, if clear solution was not obtained then 1v (with respect to apixaban) of mixture of acetonitrile and water having ratio 10:90 was kept on adding till to achieve clear solution. The solution was then concentrated under vacuum at temperature below 45°C to a volume of up to about 3 times, followed by cooling the contents to 25-30 °C. The resultant mass was diluted with water and apixaban form N-1 was isolated by filtration and dried at 50-55 °C under vacuum. The said combination of solvent not only facilitated the dissolution of apixaban in minimum volumes of solvent but also brought effective de-solvation of DMF-solvate exclusively to form N-1. Moreover, the form N-1 obtained by this process was free from form-α or form H2-2 by XRPD. The established de-solvation process for making form N-1 of 1 was found to be robust enough to deliver consistently form N-1 and surprisingly it overwhelm all the limitations of reported procedure.[11h]The PXRD, DSC and TGA of the N-1 form of apixaban obtained after de-solvation as described above are provided in Fig. 1, Fig. 2 and Fig. 3 respectively. The thermogravemetric analysis of form N-1 showed weight loss of about 0.35% confirming that it is a pure anhydrous form and DSC thermogram exhibited endotherm at about 237.39 °C.

Process for making form H2-2: As discussed in the previous section, no defined process for preparation and isolation of H2-2 form of apixaban in high chemical and polymorph purity is reported. The reported process just passes though form H2-2 (the precipitated solid in the reaction mass shows H2-2 form but further processing to isolate the H2-2 form of apixaban is not provided) with isolation of apixaban in its N-1 form.[11h]Thus, we attempted exclusive synthesis of H2-2 form by different crystallization processes using various solvents (methanol, ethanol, isopropyl alcohol, ethyl acetate, acetone, dichloromethane) and their combination. Among the explored solvent combinations, mixture of methanol and dichloromethane provided form H2-2 in the initial screening studies but failed to provide the consistent results due to formation of apixaban-DCM solvate. Remarkable improvement was achieved when methanol was replaced with acetone during the screening of different solvents and their combination. Additionally, the addition of minimum quantity of water to the mixture of acetone and DCM provided reproducibility and consistency in delivering form H2-2. The ratio of solvents played remarkable role in producing the form H2-2. After a lot of trial and error experiments the ratio of acetone: dichloromethane: water (8:4:1) mixture was fixed to provide form H2-2 consistently. Additionally to have better control on dichloromethane content wet form H2-2 was slurred in water, filtered and finally dried at 50-55°C to obtain pure apixaban (**1**) having form H2-2 without any contamination of form-α or form N-1 having dichloromethane well within the desired ICH limit. Surprisingly it was noticed that the transformation of form N-1 to form H2-2 preceded without dissolution of **1**in the identified solvent combination with excellent chemical purity and desired polymorph purity consistently. Thus we succeeded in achieving solid to solid transformation which was found to be a must criterion to achieve the transformation. During drying of form H2-2, a considerable proportion of water was often removed without disturbing the crystal network, and in fact the crystals were found to rehydrate without disturbing the crystal network when exposed to atmospheric conditions indicating that it is non-stoichiometric hydrate. The powder X-Ray diffraction pattern, DSC thermogram and TGA of the obtained apixaban form H2-2 are captured in Fig. 4, Fig. 5 and Fig. 6 respectively. Form H2-2 was crystalline in nature as witnessed by PXRD data. DSC thermogram exhibits endotherm at 94.36 °C, 232.76 °C followed by melting endotherm at 237.54°C.

Process for making form-α: Process for making form-α as per reported process using H2-2 with acetone furnished low chemical purity and further crystallization attempted to achieve the chemical purity on impure form-α led to contamination of form N-1.[11g]Thus we explored solvent screening experiments at our end to prepare form-α using from H2-2 as starting material. Surprisingly all the experiments to achieve form-α using form-N1 were unsuccessful. During initial screening, we

suspended form H2-2 in set of solvents at their reflux temperature and samples were withdrawn periodically and analyzed for XRD. Solvents acetone and acetonitrile independently furnished form-α with reproducible results without undergoing dissolution. Further volumes of acetone and reflux time required for the conversion of form H2-2 to form-α were optimized. As per the optimized process around 15-16 volumes of acetone and reflux time of 16-18 h was required for the conversion of form H2-2 to form-α. Form-α was then isolated by filtering the contents to 0-5°C followed by drying at 50-55°C to obtain pure apixaban (**1**) having form-α without any contamination of form H2-2 or form N-1.Similar to form-H2-2, form-α was also found to be a non-stoichiometric hydrate. The powder X-Ray diffraction pattern, DSC thermogram and TGA of apixaban form-α are captured in Fig.7, Fig. 8 and Fig. 9 respectively. DSC thermogram exhibits endotherm at about 50-85°C, 145-155°C, 234.27 °C followed by melting endotherm at 236.87 °C. Additionally it also showed exotherm at around 167 °C. The thermogravemetric analysis of form-α showed loss of 3.21% confirming that it is also a non-stoichiometric sesquihydrate

Study of polymorphic transformations:

Information on polymorphic transformation in different solvents at different temperature and at different intervals provided good insight to set the process for development and manufacturing the product under cGMP conditions. It can be used as an important tool for the assessment of changes in physical form of drug molecules. Thus, with the available polymorphs (Form H2-2, Form-αand Form N-1) we evaluated their changes in different solvents at reflux temperature and at different intervals to understand their stability (Table 3). Form N-1 remained unchanged in all the solvents at different temperature and time intervals whereas H2-2 or form-α showed remarkable transformations in different solvents. Alcoholic solvents tend to convert both form H2-2 and form-α to form N-1 whereas acetone and acetonitrile ~~were~~ tends~~ing~~ to convert form H2-2 to form-α. Water as a solvent tends to convert form-α to form H2-2 (Table 2) till 6 to 7 hours. Further heating of form-α in water leads to complete transformation to form N-1. Thus, over all study reveals that form N-1 is the most stable form of apixaban. Polymorphic transformations studied are systematically represented in fig.10.

CONCLUSIONS

This communication describes the investigation on the polymorphic forms of apixaban (**1**), an anticoagulant agent associated with basic synthetic process. This article presents efficient and impurity free process for the preparation of apixaban with high chemical and polymorph purity. Process optimization details to understand the critical parameters which are responsible to provide the stable polymorphs at a scale are discussed. The systematic study conducted to achieve polymorph transformations is also presented.

ACKNOWLEDGEMENTS

The authors are grateful to the management of Megafine Pharmaceuticals (P) Ltd., for supporting this work. We also thank colleagues of Analytical Research and Development Department, Megafine Pharmaceuticals (P) Ltd., for valuable cooperation in developing the chromatographic methods for establishing the process and identifying the impurities.

Table 1. Solubility profile of apixaban (1) in various solvents

Solvent	Solubility Criteria[14]						
	I	II	III	IV	V	VI	VII
Water	X	X	X	X	X	X	√
Ethanol	X	X	X	X	√	-	-
Methanol	X	X	X	X	√	-	-
Dimethylformamide	X	X	√	-	-	-	-
Ethyl acetate	X	X	X	X	X	X	√
Dimethylsulphoxide	X	X	X	√	-	-	-
Chloroform	X	X	X	X	√	-	-
Acetone	X	X	X	X	X	X	√
Dichloromethane	X	X	X	√	-	-	-
Isopropyl alcohol	X	X	X	X	X	X	√
Toluene	X	X	X	X	X	X	√
THF	X	X	X	X	X	√	-
Acetonitrile	X	X	X	X	√	-	-
Acetic acid	X	√	-	-	-	-	-
N-Methyl-2-pyrrolidone	X	√	-	-	-	-	-
Dimethylacetamide	X	√	-	-	-	-	-

(Notes: X= do not meet criteria; √ = meets the criteria).

Table 2. Solvent optimization details to achieve form N-1:

Sr. No	Ratio of Ethanol: Acetonitrile: water	Total volume	Remarks
1	10v: 1.5v: 1.5v	13	Form N-1 with higher content of Acetonitrile
2	10v: 1.0v: 2.0v	13	Form N-1 with higher content of Acetonitrile
3	10v: 1.5v: 3.5v	15	Form N-1 with higher content of Acetonitrile
4	10v: 0.7v: 6.3v	17	Form N-1 with acceptable limit of acetonitrile
5	10v: 0.3v: 2.7v	13	Form N-1 with acceptable limit of acetonitrile

Table 3: Details of polymorph transformations in different solvents at reflux temperature

Sr. No	Solvent used	Polymorph used for the test	Polymorph obtained after 3.0 h. reflux	Polymorph obtained after 6.0 h. reflux	Polymorph obtained after 12h. reflux
1	Ethanol	H2-2 or Form-α.	Form N-1	Form N-1	Form N-1
2	Methanol	H2-2 or Form-α.	Form N-1	Form N-1	Form N-1
3	Isopropanol	H2-2 or Form-α.	Form N-1	Form N-1	Form N-1
4	Acetone	H2-2 or Form-α.	form-α	form-α	form-α
5	Acetonitrile	H2-2 or Form-α.	form-α	form-α	form-α
6	Ethyl acetate	H2-2 or Form-α.	Form N-1	Form N-1	Form N-1
8	Methyl tertiary butyl ether	H2-2 or Form-α.	Form N-1	Form N-1	Form N-1
9	Water (at 75-80° C)	H2-2 or Form-α.	form H2-2	form H2-2	form N-1

Figure 1: PXRD pattern of N-1 form apixaban obtained after de-solvation.

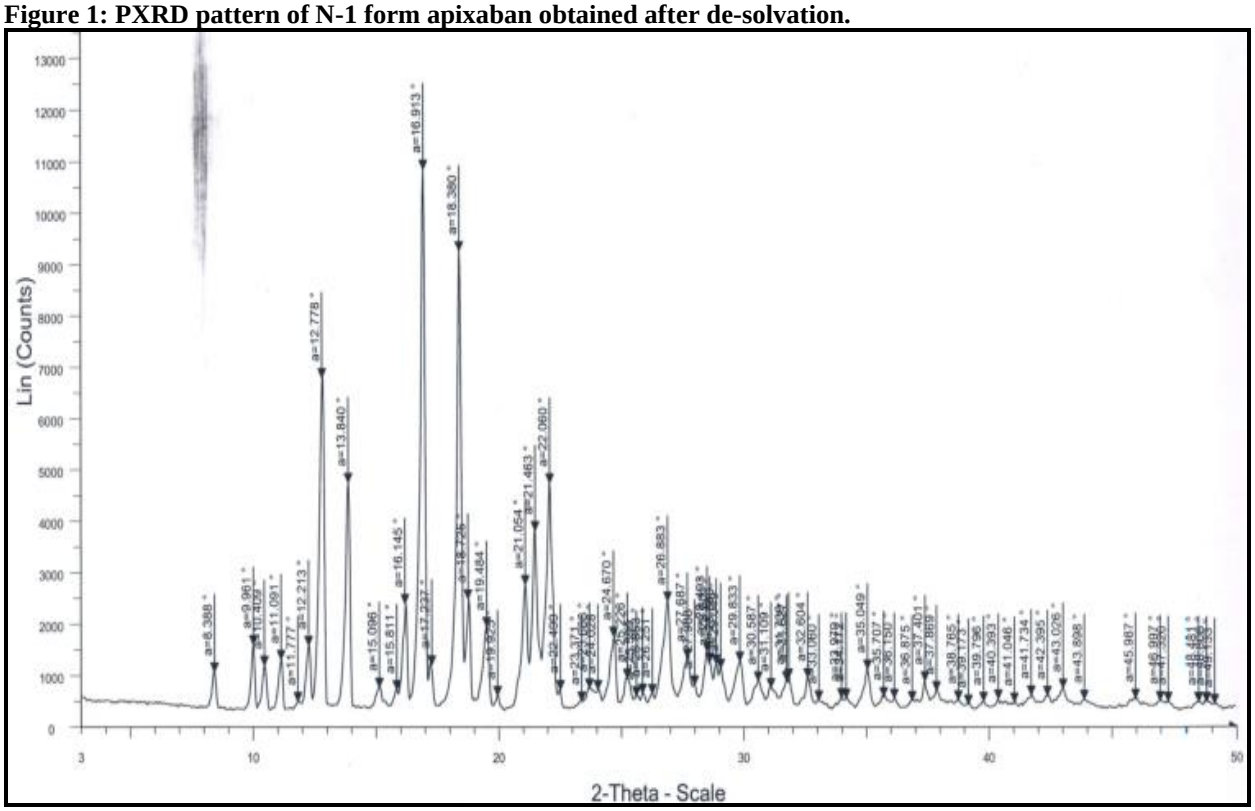


Figure 2: DSC thermogram of N-1 form of apixaban obtained after de-solvation

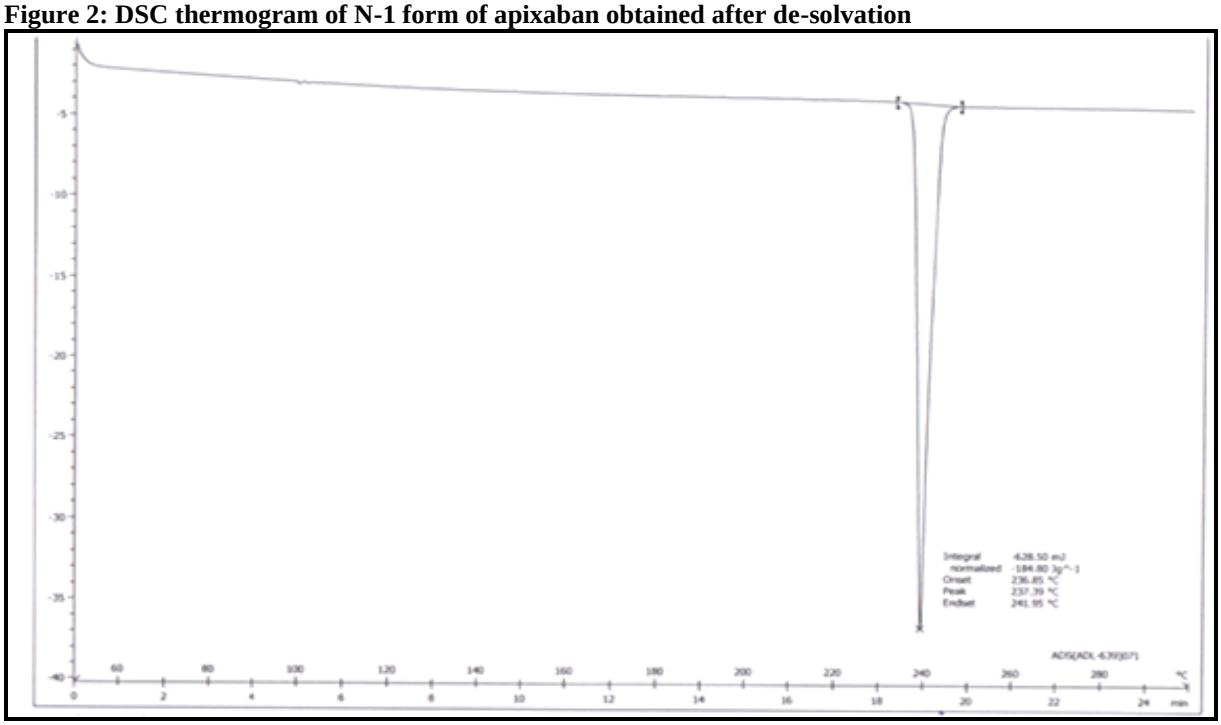


Figure 3: TGA of N-1 form of apixaban obtained after de-solvation.

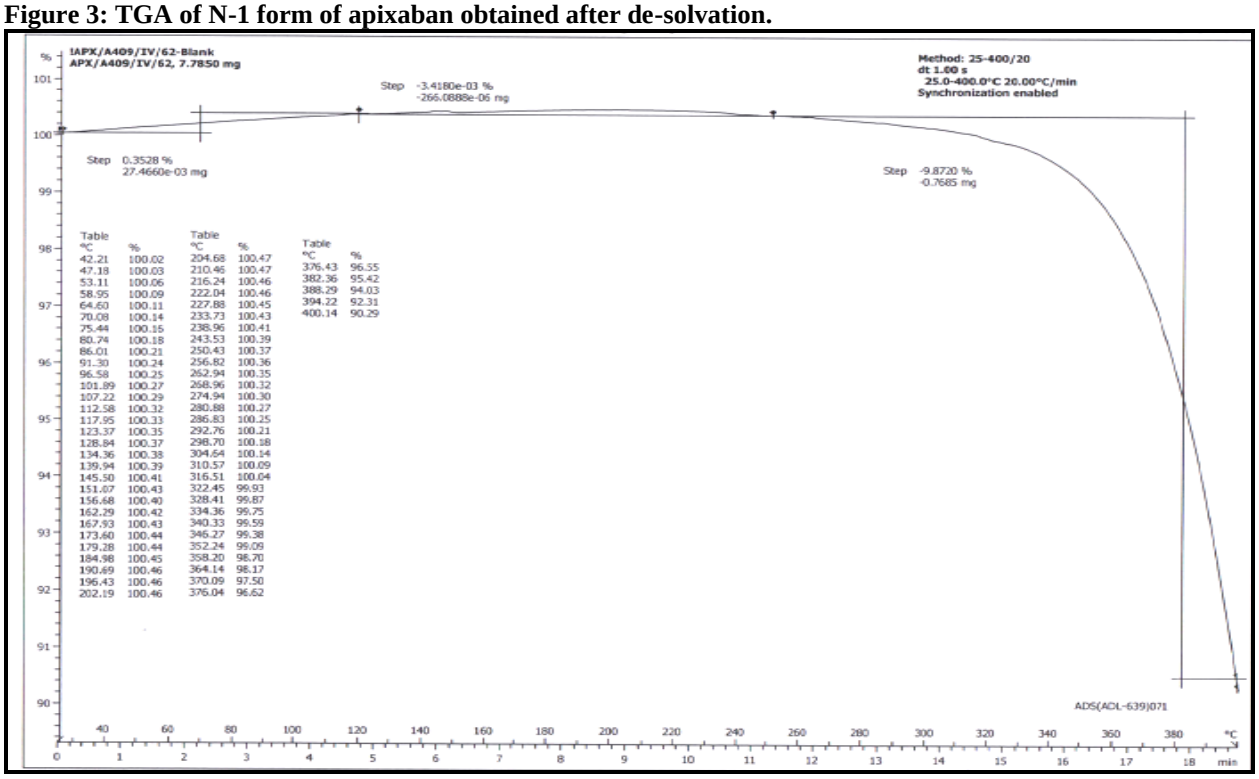


Figure 4: Powder X-Ray diffraction pattern of apixaban form H2-2.

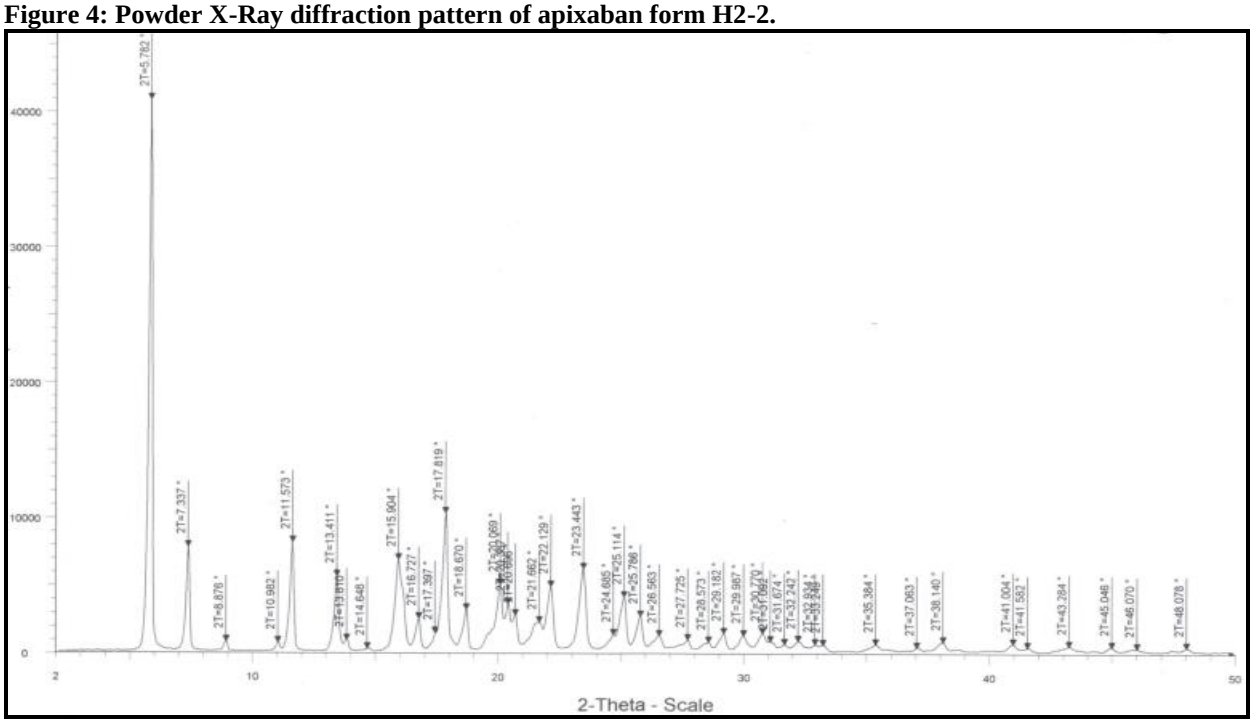


Figure 5: DSC thermogram of apixaban form H2-2

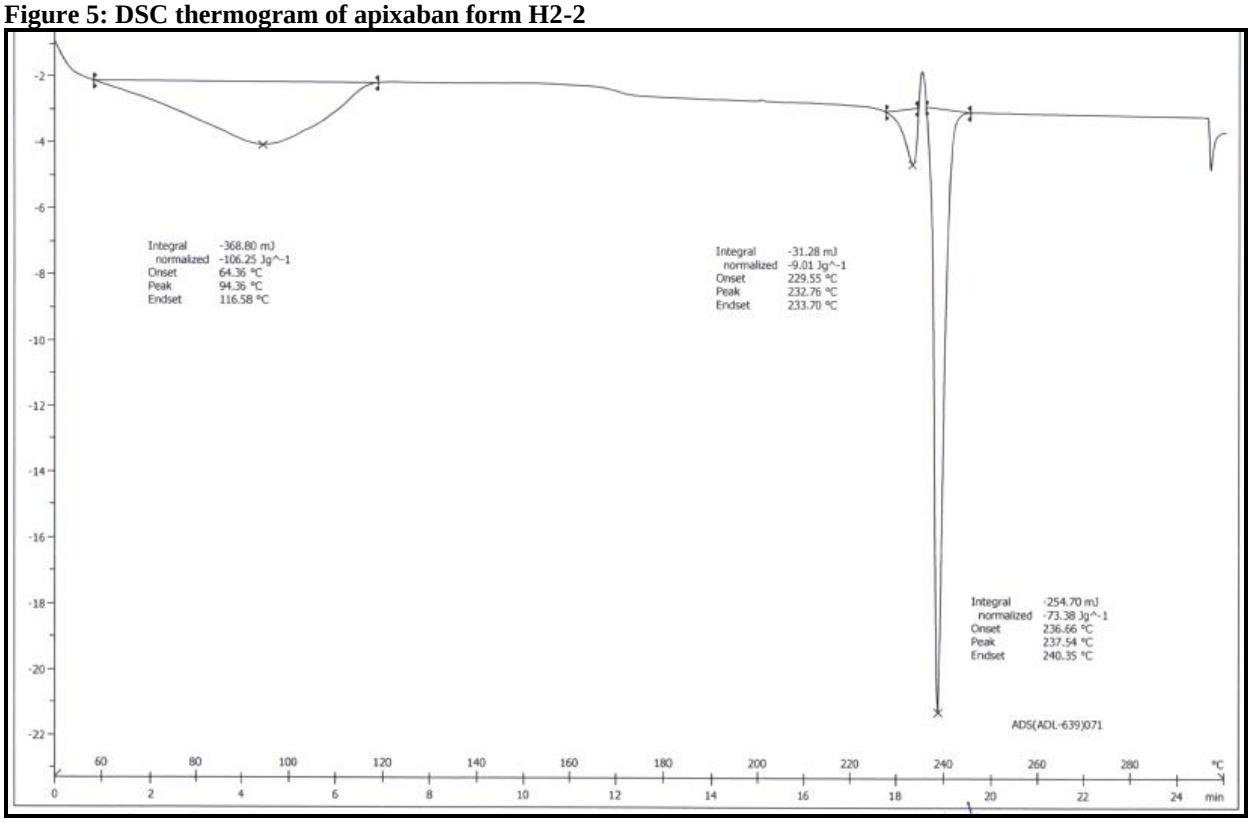


Figure 6: TGA of apixaban obtained form H2-2

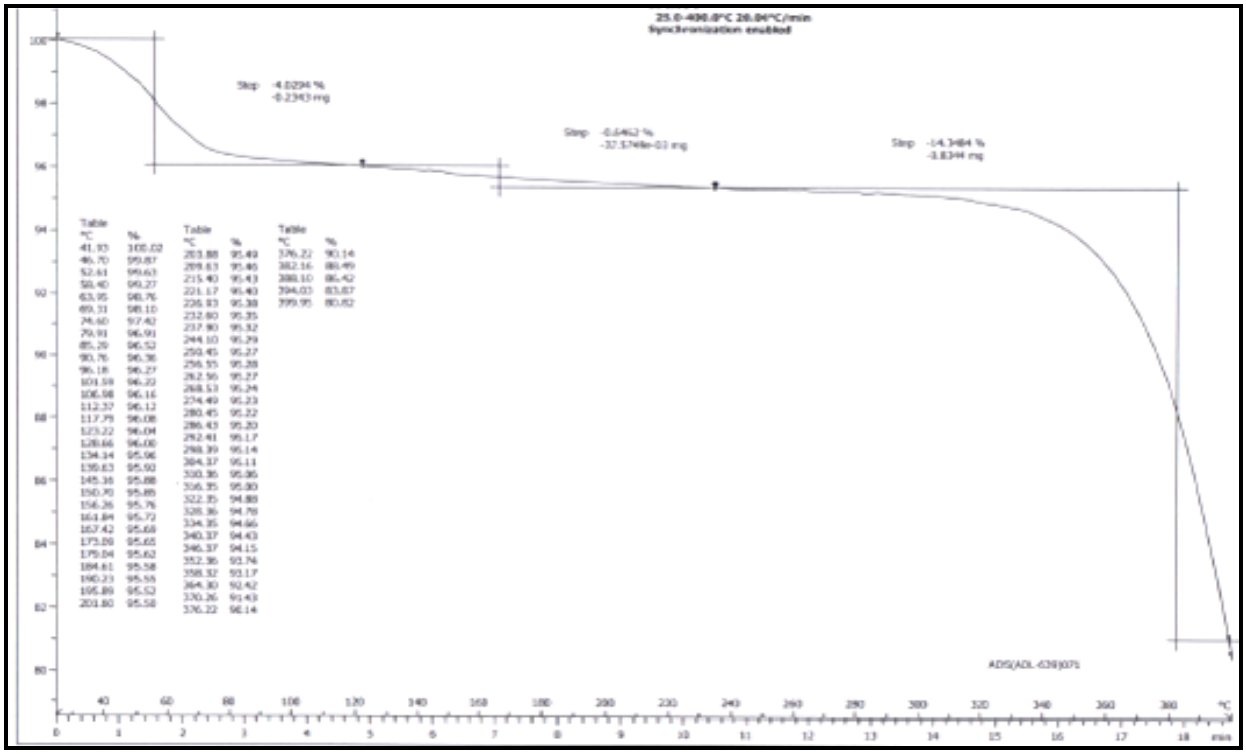


Figure 7: Powder X-Ray diffraction pattern of apixaban form-*α*.

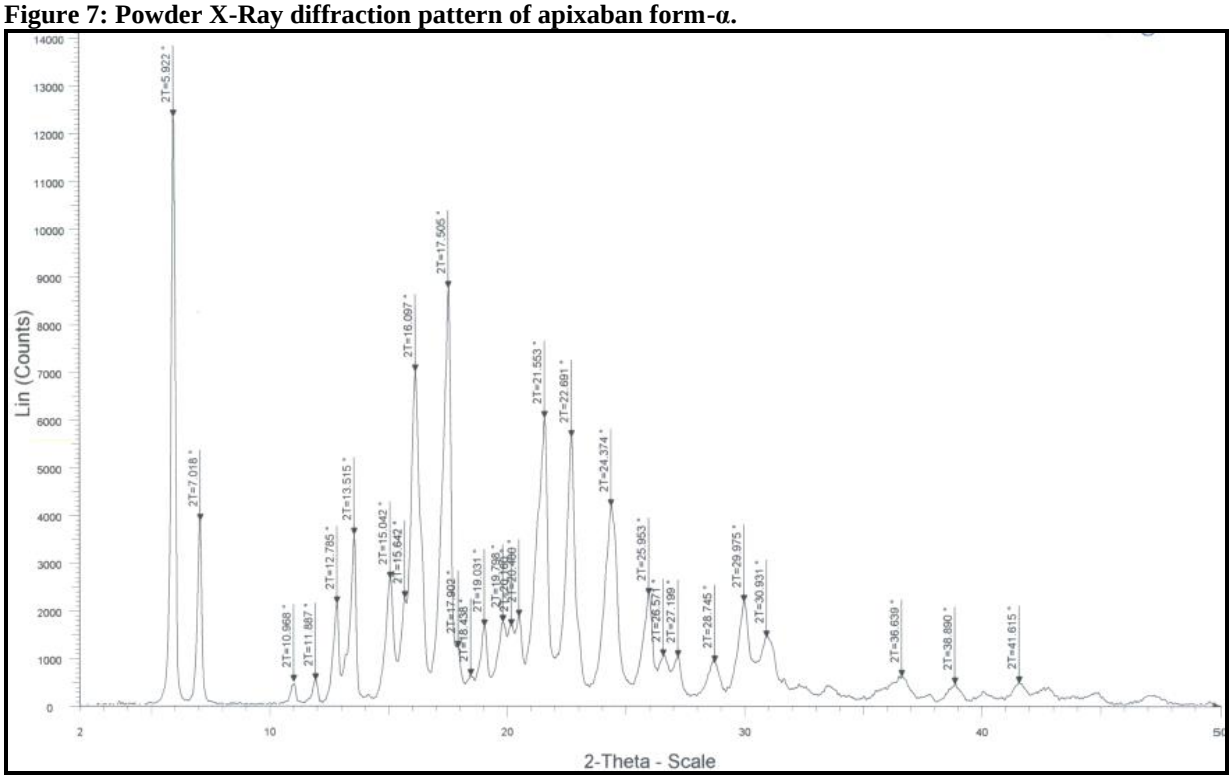


Figure 8: DSC thermogram of apixaban form-α

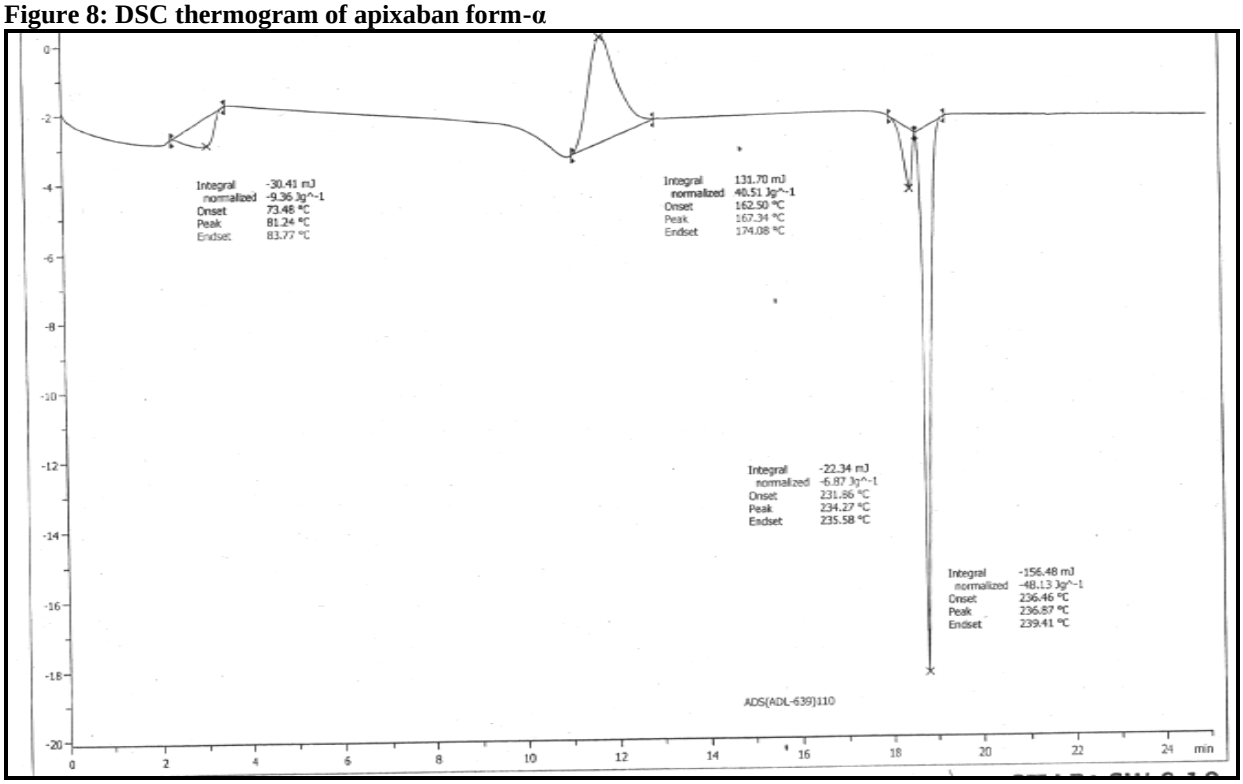


Figure 9: TGA of apixaban form-α

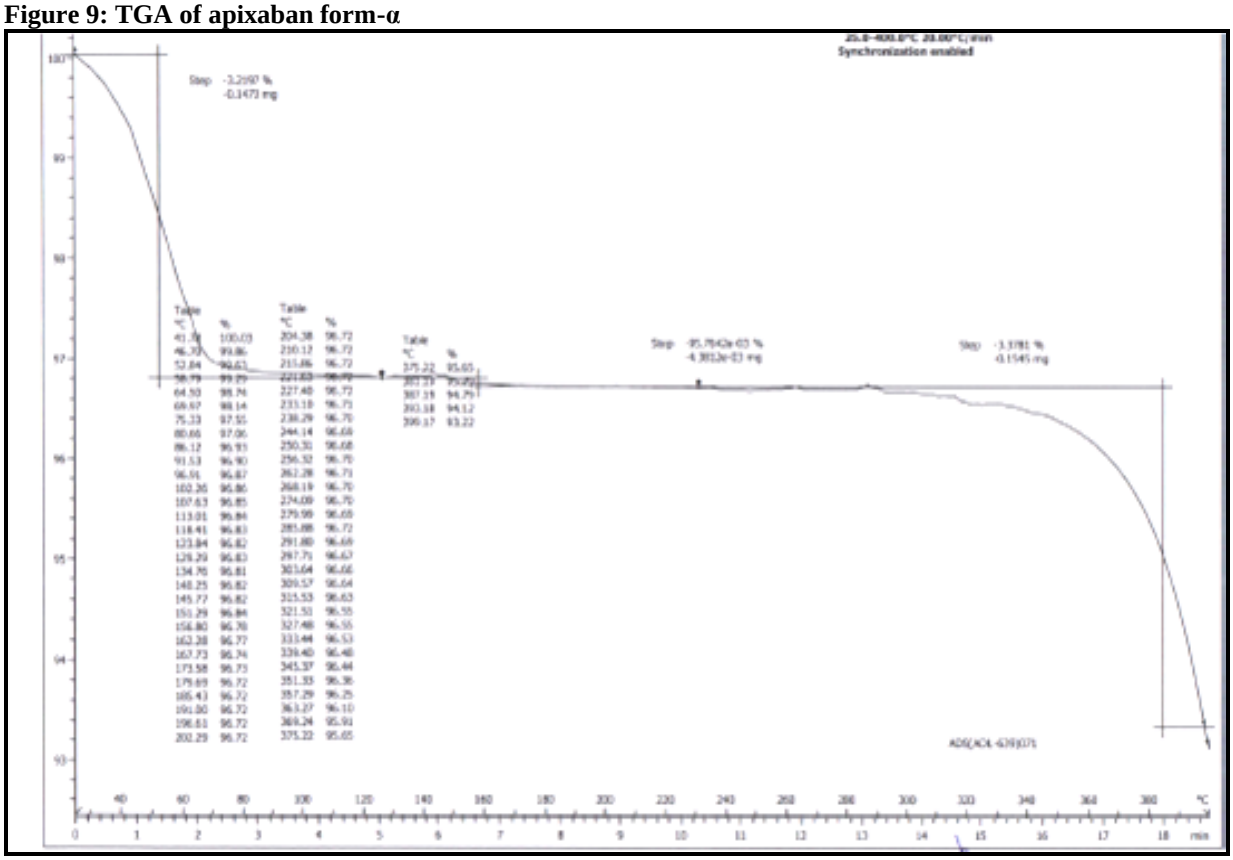
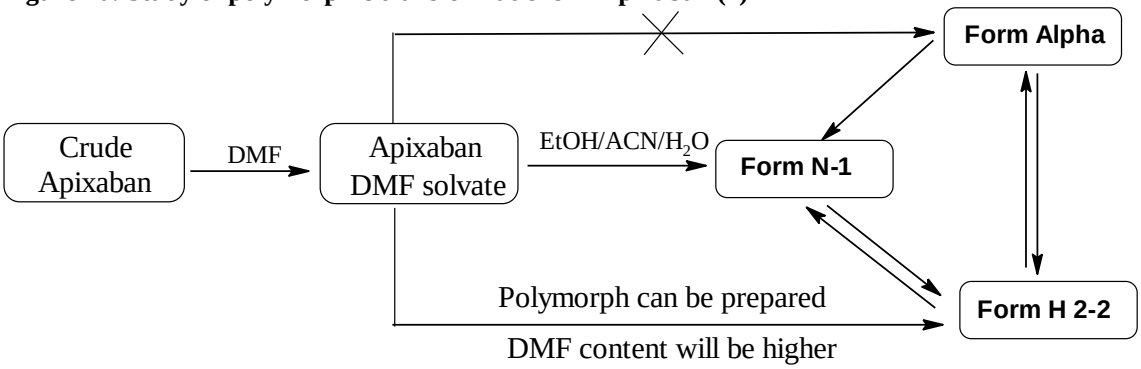


Figure 10: Study of polymorphic transformations in Apixaban (1)



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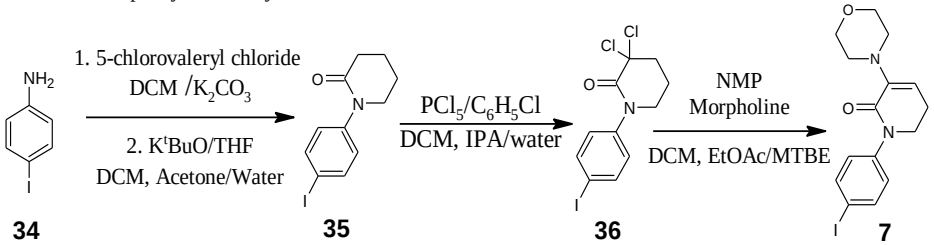
12. All the operations have to be performed under anhydrous conditions and under nitrogen atmosphere.
13. Synthesis of key intermediate **7** was achieved by acylation of 4-Iodoaniline (**34**) with 5-chlorovaleryl chloride in DCM in the presence of K_2CO_3 as a base at 10-15°C for 3-4 h. Acylated product was then cyclized *in-situ* after work-up using K-*t*BuO as cyclizing agent in THF to providepiperdin-2-one derivative (**35**)which was extracted in DCM and crystallized using acetone-water with an overall yield of 84% and purity of 99.5% by HPLC. Chlorination of **35** was done using PCl_5 in chlorobenzene, obtained crude **36** was extracted in DCM and crystallized using isopropyl alcohol-water with an yield of 78% and purity of 99.45%.Condensation of obtained dichloro compound (**36**) with morpholine provided**7** as a crude solid which was purified from ethyl acetate and methyl-*tert*-butylether (MTBE) with an yield of 75% and purity of 99% by HPLC



14. Solubility matrix presented was measured as per United States Pharmacopeia (USP) standard procedure mentioned in USP 37, NF 32, general notice, page no. 6.