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Original Article

FORMULATION AND EVALUATION OF TABLET PREPARED BY COAMORPHOUS SYSTEM CONTAINING ANTI-HYPERTENSIVE AND ANTI-HYPERLIPIDEMIC DRUG

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ABSTRACT

Objective: Amlodipine besylate (AML) and Atorvastatin calcium (ATR) belong to biopharmaceutical classification system (BCS) class II (i.e. low solubility and high permeability) which leads to variable bioavailability. Hence, the aim of this study was to enhance the solubility of both drugs by utilizing the co-amorphous technique. This converted form and physical mixture of both drugs were utilized in the formulation of tablets.

Methods: The co-amorphous system was prepared by using rotary flash evaporator. Solubility study was carried out to investigate the dissolution advantage of prepared co-amorphous form. Total twelve formulations were formulated by keeping constant drugs concentrations utilizing direct compression technique among which F1 to F6 contains co-amorphous AML-ATR (Co-A AML-ATR) and F7 to F12 contains a physical mixture of AML and ATR as active pharmaceutical ingredient (APIs). Pre-compression and post-compression studies were carried out to all twelve formulations. Stability study was performed to the optimized formulations as per ICH guidelines.

Results: Mixture obtained after evaporation was found to become amorphous. FTIR study shows no evidence of intermolecular interactions between AML and ATR. The solubility of both AML and ATR were increased in almost one fold as compared to their respective crystalline counterparts. Pre-compression parameters of all twelve formulations blend fall under excellent to fair to flow properties. Post-compression parameters of all twelve formulations were within the specifications. But *in vitro* drug release of formulations F5, F6, F11, and F12 showed % drug release as per IP. Stability study of optimized formulations was observed with, no significant difference in % drug release.

Conclusion: The co-amorphous system can be prepared by utilizing rotary flash evaporator and the same was confirmed by XRPD and FTIR studies. The dissolution rate of the co-amorphous system was greater than that of the crystalline counterpart. Based on the results; F5 and F6 are considered as optimized formulations. Optimized formulations were stable during the stability study.

Keywords: Amlodipine besylate, Atorvastatin calcium, the Co-amorphous system of AML-ATR, Rotary flash evaporator, Direct compression, Sodium starch glycolate, Crospovidone, and Stability study

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INTRODUCTION

Early trials on the treatment of hypertension based upon high doses of diuretics or beta blockers demonstrated a significant reduction in stroke but a less prominent decline in the incidence of coronary heart disease. This relative lack of coronary benefit may have been related to the adverse effect of these antihypertensive agents on plasma lipids since even small elevations in serum cholesterol may significantly increase coronary risk, particularly in patients with underlying hypertension. Drug-induced changes in lipid levels may be particularly important in hypertensive since up to 40 percent of untreated patients with primary hypertension (formerly called "essential" hypertension) already have lipid abnormalities, such as a high LDL-cholesterol and a low HDL-cholesterol.

Hence, hypertension frequently coexists with hyperlipidemia and both are considered to be major risk factors for developing cardiac disease ultimately resulting in adverse cardiac events. This clustering of risk factors is potentially due to a common mechanism. Further, patient compliance with the management of hypertension is generally better than patient compliance with hyperlipidemia. It would, therefore, be advantageous for patients with the intent of providing commercially viable multi-dose combinations in tablet dosage form to have a single therapy which treats both these conditions and minimizing tablet weight to assure patient acceptance [1, 2].

In this regard many combination treatments have come into the market namely, amlodipine besylate with atorvastatin calcium, telmisartan with atorvastatin, olmesartan with atorvastatin, simvastatin with aspirin with lisinopril with atenolol, atorvastatin calcium with metoprolol tartrate [3]. Among these available combinations, we opted combinational drug therapy as amlodipine besylate (AML) and atorvastatin calcium (ATR). This combination is bioequivalent to amlodipine and atorvastatin gave alone and does not modify the efficacy of either single agent, therapeutically shows a synergistic antioxidant effect on lipid peroxidation in human low-density lipoproteins and membrane vesicles enriched with polyunsaturated fatty acids, whereby these synergistic combinations are useful in treating subjects suffering from angina pectoris, atherosclerosis, combined hypertension and hyperlipidemia and those subjects presenting with symptoms of cardiac risk, including human. By utilizing this combination as an advantage on unconscious and unresponsive hypertensive patients thereby improving patient compliance, decreasing the cost of the treatment and number of pills [4, 5].

AML and ATR are one of the most frequently prescribed drug combinations in the world and oral route of choice for the drug administration due to its ease of administration, high patient compliance, cost-effectiveness, least sterility constraints and flexibility in the design of dosage form. But the major problem with both AML and ATR is 'low aqueous solubility'. The term 'low aqueous solubility' means that the drug has a solubility of less than about 10 mg/ml, and preferably less than about 5 mg/ml, in aqueous media at approximately physiological temperatures and pH. The bioavailability of these drugs can be limited by poor dissolution of the drug into aqueous bodily fluids the following administration. This rate-limiting step may, therefore, be critical to rapidly attaining therapeutically effective amlodipine and atorvastatin drugs levels, inadequate and variable bioavailability and gastrointestinal mucosal toxicity [6-10]. A number of novel approaches for enhancing the low aqueous solubility of drugs have been attempted and continued to evolve over a period. Reduction in particle size (nano-drug delivery) and increased surface area, the use of alternative salt forms,

solubilization of drug in co-solvents or micellar solutions, complexation with cyclodextrins or the use of lipid-based vehicles for the delivery of lipophilic drugs to name few. Among which co-amorphous system also is an alternative and novel approaches for the enhancement of solubility. In these systems, a combination of two small molecules (drugs or excipients) is used instead of drug–polymer mixtures. These systems have been found to provide high stability and enhanced dissolution rates for the drugs. There are several publications in the area of amorphous binary systems like ritonavir with indomethacin, naproxen with cimetidine, simvastatin with glipizide, and atorvastatin with nicotinamide [11-13].

With this background, the present study was undertaken to develop a formulation containing co-amorphous forms of drugs AML and ATR useful for a population with the co-morbid condition of hypertension and hyperlipidemia.

MATERIALS AND METHODS

Chemicals and reagents

Amlodipine besylate and atorvastatin calcium are the gift samples from Dr. Reddy's Laboratories limited, Telangana (India). Methanol purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. Sodium starch glycolate (SSG), crospovidone (CP), microcrystalline cellulose, colloidal silicate and magnesium stearate were purchased from Sigma-Aldrich Corporation, Bengaluru, India. Potassium dihydrogen phosphate and potassium bromide (IR grade) were purchased from Merck, Mumbai, India. All other reagents were of analytical grade.

Methods

Melting point

The melting point of AML and ATR was determined by taking a small amount of both the drug in separate capillary tubes were closed at one end and placed in a melting point apparatus and the temperature at which drug melts was recorded. This was performed in triplicates and the average value was noted.

Solubility study

The solubility studies were performed in phosphate buffer pH 6.8, by adding excess amounts of AML, ATR, and co-amorphous system in each case to form saturated solution and keeping flasks on a rotary shaker with an agitation speed of 200 rpm and temperature controlling system of±0.1 °C for 24 h. After 24 h, solutions were filtered through 0.45 µm filters and diluted with phosphate buffer pH 6.8 and analyzed using UV spectrophotometer (Shimadzu UV-1800, Japan) at 365 nm and 241 nm for amlodipine besylate and atorvastatin calcium respectively, which are the absorption maxima's determined earlier and drug concentrations were calculated.

FT-IR Study [14, 15]

This was carried out to find out the compatibility between the physical mixture of AML and ATR, prepared co-A AML-ATR and AML-ATR mixtures with polymers such as sodium starch glycolate, crospovidone, microcrystalline cellulose, colloidal silicate, and magnesium stearate. 10 mg of the sample and 400 mg of KBr were taken in a mortar and triturated. A small amount of the triturated sample was taken into a pellet maker and was compressed at a 10 kg/cm^2 hydraulic press. The pellet was kept onto the sample holder and scanned from 4000 cm^{-1} to 600 cm^{-1} in FT-IR spectrophotometer (Shimadzu FTIR-8700). The spectra obtained were compared and interpreted for the functional group peaks.

Preparation of amorphous precipitate of AML–ATR binary system [11, 13]

The co-amorphous system of AML and ATR were prepared by solvent evaporation technique using methanol as a solvent. A total of 1500 mg in 1000 mg of atorvastatin calcium and 500 mg of amlodipine besylate were mixed homogeneously and then dissolved in 20 ml methanol. The solvent was evaporated under reduced pressure at 40 °C. The residual solvent left after evaporation was then removed completely by placing the sample under vacuum for 2 d inside desiccator containing $CaCO_3$. The precipitates were stored in a desiccator until its use in the experiment.

X-ray powder diffractometry (XRPD) [16]

X-ray powder diffraction patterns were obtained using Rigaku miniflex 600 X-ray diffractometer (Rigaku Co., Tokyo, Japan) for pure AML, ATR and co-amorphous form of AML-ATR (co-A AML-ATR). The instrument was operated at 600 watts (X-ray tube), with a fixed tube current of 15 mA and a voltage of 40 kV. The diffracted X-ray beam was monochromated by a graphite monochromator and a standard scintillation counter was used as the detector. Diffraction intensities were measured by fixed time step scanning method in the range of 0–50 ° (2θ).

Preparation of tablets containing physical mixture of ATR and AML and co-A AML-ATR by direct compression

All the solid raw materials were dispensed, packed in an individual in clean poly bags and labeled. Separately weighted quantities as given in table 1 of formulation F1 to F6 containing of co-A AML-ATR, sodium starch glycolate, crospovidone, and microcrystalline cellulose sifted through #30 mesh and colloidal silicate and magnesium stearate through #60 mesh. All the above-sifted materials are collected individually into a double-lined polyethylene bag. The co-amorphous system of AML-ATR, crospovidone XL, sodium starch glycolate, and microcrystalline cellulose was mixed in a mortar and pestle for 10 min. To the premixed blend, colloidal silicate was added and thoroughly mixed for 15 min. Lubricate the above blend with magnesium stearate and hand blend (mix) for 5 min. The tablets were prepared using 8 mm Flat Faced Bevel Edged (FFBE) punches. The tablets were compressed by maintaining a constant hardness 7±0.5 kg/cm^2.

For the formulation F7 to F12 of the tablets containing a physical mixture of AML and ATR, similar steps were followed and quantities are given in table 2.

Table 1: List of materials and quantities for formulations F1 to F6

Name of the ingredients	Quantity/Unit dose (mg)					
	F1	F2	F3	F4	F5	F6
Co-A AML-ATR (5 mg of AML and 10 mg of ATR)	15	15	15	15	15	15
Sodium starch glycolate	5	-	10	-	15	-
Crospovidone	-	5	-	10	-	15
Microcrystalline cellulose	175	175	170	170	165	165
Colloidal silicate	2	2	2	2	2	2
Magnesium stearate	3	3	3	3	3	3
Weight of the tablet (mg)	200	200	200	200	200	200

Pre-compression parameters [17-19]

Pre-compression parameters like Bulk density (Db), Tapped density (Dt), Compressibility index (Carr's Index), Hausner's ratio and Angle of repose were performed to all formulation blends.

Post-compression parameters [14, 20-25]

Thickness

The thickness and diameter of the tablet were measured using Vernier calipers. It is measured in mm.

Table 2: List of materials and quantities for formulations F7 to F12

Name of the ingredients	Quantity/Unit dose (mg)					
	F7	F8	F9	F10	F11	F12
AML	5	5	5	5	5	5
ATR	10	10	10	10	10	10
Sodium starch glycolate	5	-	10	-	15	-
Croospovidone	-	5	-	10	-	15
Microcrystalline Cellulose	175	175	170	170	165	165
Colloidal silicate	2	2	2	2	2	2
Magnesium Stearate	3	3	3	3	3	3
Weight of the tablet (mg)	200	200	200	200	200	200

Weight variation test

20 tablets were selected at random from the lot, weighed individually and the average weight was determined. The percent deviation of each tablets weight against the average weight was calculated. The test requirements are meets, if not more than two of the individual weights deviate from the average weight by not more than existing 7.5%.

Hardness

The prepared tablets were subjected to hardness test. It was carried out by using hardness tester and expressed in kg/cm^2.

Friability (F)

The friability was determined using Roche friabilator and expressed in percentage (%). 10 tablets from each batch were weighed separately ($W_{initial}$) and placed in the friabilator, which was then operated for 100 revolutions at 25 rpm. The tablets were weighed (W_{final}) and the percentage friability was calculated for each batch by using the following formula.

$$\%\text{Friability} = \frac{\text{Initial weight} - \text{Final kweight}}{\text{Initial weight}} \times 100$$

Uniformity of drug content

The prepared tablets were tested for their drug content. 20 tablets of each formulation were finely powdered; weight equivalent to 5 mg of AMD and 10 mg of ATR and the same was completely extracted with methanol by sonication for 10 min in a 100 ml volumetric flask and this solution was filtered through Whatman no.1 filter paper. The residue was washed with 10 ml methanol three times and volume made up to 100 ml with methanol. The solution obtained was diluted with the Methanol so as to obtain a concentration in the range of linearity previously determined. The concentration of both AMD and ATR were determined by measuring the absorbance of the sample at 365 nm and 241 nm respectively.

Disintegration time

The *in vitro* disintegration time of a tablet was determined using disintegration test apparatus as per I. P. specifications. One tablet is placed in each of the 6 tubes of the basket. The experiment was done by using Phosphate buffer pH 6.8 maintained at 37±2 °C as the immersion liquid. Assembly raised and lowered between 30 cycles. The time taken for the tablet to complete disintegration with no palpable mass remaining in the apparatus was measured was recorded in s.

Water absorption ratio

A piece of tissue paper folded twice was placed in a small Petri dish Containing 6 ml of water. A tablet was put on the tissue paper and allowed to completely wet. The wetted tablet was then weighted. Water absorption ratio, R was determined using following equation.

$$R = \frac{Wa - Wb}{Wa} \times 100$$

Where,

Wa = Weight of tablet after water absorption

Wb = Weight of tablet before water absorption

Wetting time

In wetting time a piece of tissue paper folded twice was placed in a small petri dish (internal diameter = 6.5 cm) containing 10 ml of 5% amaranth solution, a tablet was placed on the paper, and the time for complete wetting was measured. Three trails for each batch were performed and the standard deviation was also determined.

***In vitro* drug release**

The *in vitro* dissolution studies were carried out for the formulations using USP apparatus type II (Paddle type). The dissolution medium used was 900 ml of phosphate buffer of pH 6.8 for 30 min. The temperature was maintained at 37 °C±0.5 °C and the stirring rate was 50 rpm. 5 ml of samples were withdrawn at intervals of 5 min up to 30th min; the same volume was replaced with freshly prepared pH 6.8 phosphate buffer. The samples were measured by UV Spectrophotometer at 365 nm and 241 nm against blank for AML and ATR respectively. The release studies were conducted in triplicate and the mean values were plotted versus time.

Stability study [26]

The selected formulations were packed in a suitable container, which mimics the final packing. They were then stored at 25 °C±2 °C/60%±5% RH and 40 °C±2 °C/75%±5% RH and kept for three months and evaluated for their hardness, drug content, and drug release at specific intervals of time as per ICH Guidelines.

Statistical analysis

One way ANOVA followed by Tykey method opted as a statistical analysis of obtained *in vitro* release data. A probability value of $p<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Melting point

Melting point analysis is one of the quality control tests. The melting point of pure AML and ATR were given in below table 3. By comparing reported melting point with an actual melting point which are in the range of IP specification and have sharp melting point hence, both the drugs which are of genuine and crystalline in nature.

Table 3: Melting point of AML and ATR

Name of the drug	Melting point	
	Actual	Reported*
AML	178-179 °C	178.33 °C±0.3055
ATR	159.2-160.7 °C	160.33 °C±0.1155

*mean±SD (n=3)

Solubility studies

The solubility of pure drugs and prepared co-amorphous system were showed in the graphical representation in fig. 1 and 2. The saturation solubilities of crystalline AML and ATR in phosphate buffer (pH 6.8 at 37 ℃) were 357.61 µg/ml and 100.14 µg/ml, respectively. A statistically significant enhancement, i.e., almost one fold in the saturation solubilities of the individual components as compared to their crystalline counterparts were reported in the amorphous binary system these findings are similar as reported by A Shayanfar *et al.*, 2013 and S. J. Dengale *et al.*, 2014.

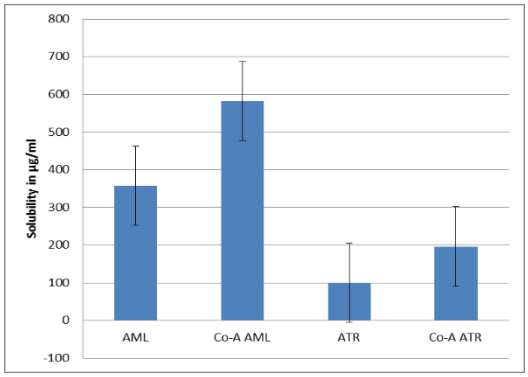

Fig. 1: Solubility representation in column chart of both AML and ATR in crystalline and co-amorphous form

Drug-excipients compatibility studies

FTIR spectroscopic studies were showed in table 4 and fig. 2, 3, 4, 5, 6, and 7. FTIR measurements were carried out to pure AML, ATR, a physical mixture of ATR-AML and with formulation blend and co-A AML-ATR and with formulation blend to in order to find out the interactions. AML showed strong peaks at 3297, 3158-2982, 1676, 1614-1493, 1207, 1095, 995, and 755-730 cm^{-1} which attribute N-H stretching, C-H stretching, C=O stretching, aromatic C=C stretching, C-O stretching and S=O stretching, C-O-C stretching, C-Cl stretching, and aromatic C-H bending respectively. Whereas for ATR at 3381, 2992-2900, 3670, 3055, 1662, 1595-1531, 1157, 1213, 1224 and 843-753 cm^{-1} for N-H stretching, C-H stretching, free O-H stretching, O-H stretching, C=O stretching, aromatic C=C stretching, C-N stretching, C-O stretching, C-F stretching, and aromatic C-H bending.

In order to detect the interaction between the two drugs, the individual spectrum of each drug was compared with the spectrums that of a physical mixture of AML-ATR, co-A AML-ATR separately and with formulation blend. The results of FTIR showed that the samples comply with FTIR spectrum given in the BP. It also concludes that there are no molecular interactions in drugs and with formulation blend and in co-A ATR-AML and with its formulation bled. In co-amorphous form, there is a decrease in intensities of all the peaks, broadening and slight shifts (i.e. minor hypsochromic) are observed for N-H and C-H functional groups, which is due to the hydrogen bonding between both the drugs due to the amorphous convertion. Hence, all the reports conclude that we can proceed with the formulation of tablet.

Table 4: Compatibility study of drugs with formulation blend

Drug/Formulation blend	Drug/formulation blend ratio	Physical description initial	40 °C±2 °C/75%±5% RH		
			1st weak	2nd weak	4th weak
Amlodipine besylate	-	White crystalline powder	*	*	*
Atorvastatin calcium	-	White crystalline powder	*	*	*
AML: ATR	1: 1	White crystalline powder	*	*	*
AML: ATR: Blend	1: 1: 1	White crystalline powder	*	*	*
Co-A ATR-AML	-	White crystalline powder	*	*	*
Co-A ATR-AML: Blend	1:1	White crystalline powder	*	*	*

*No incompatibility problem

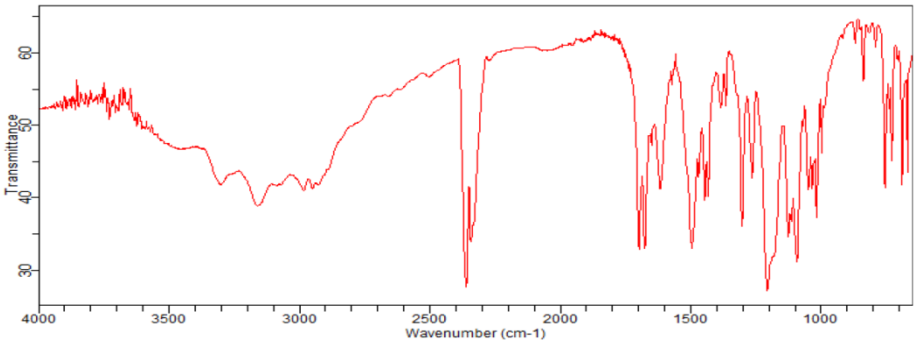

Fig. 2: FTIR spectra of AML

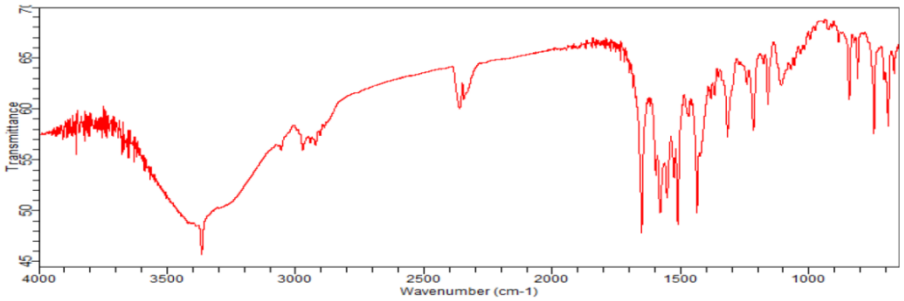

Fig. 3: FTIR spectra of ATR

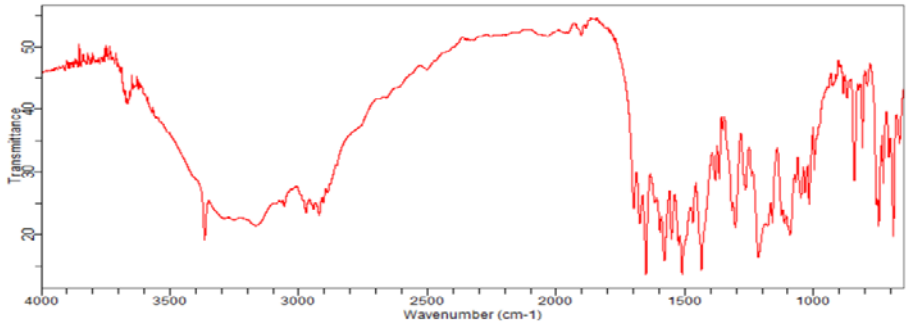

Fig. 4: FTIR spectra of physical mixture of AML and ATR

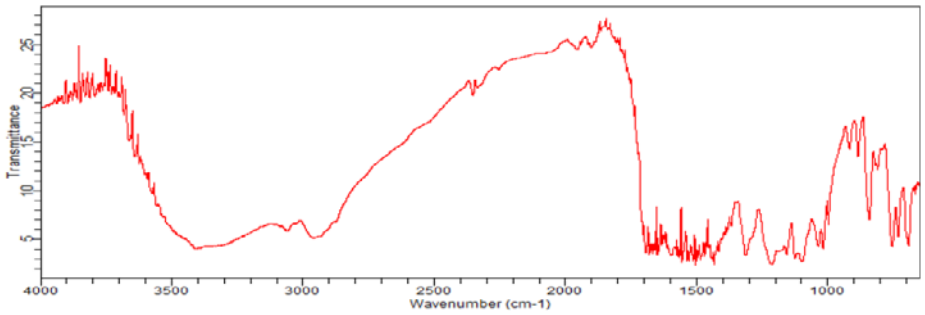

Fig. 5: FTIR spectra of co-A AML-ATR

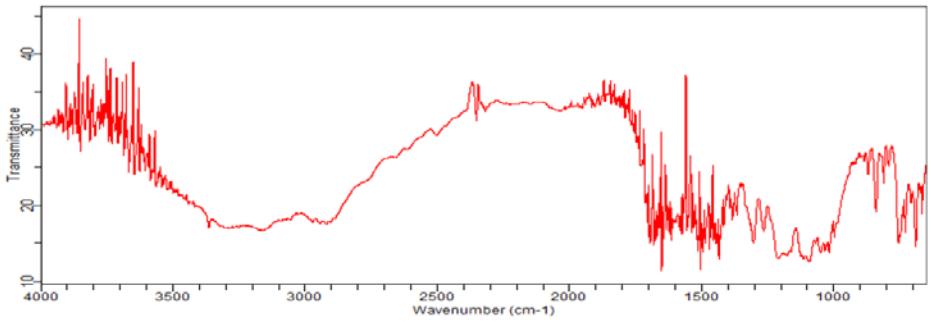

Fig. 6: FTIR spectra of physical mixture AML-ATR with formulation blend

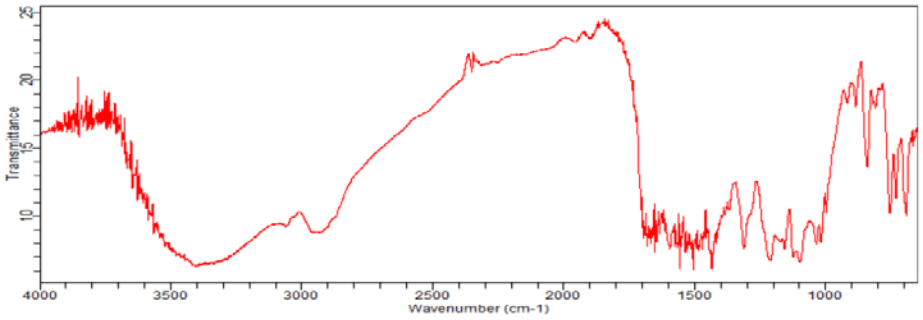

Fig. 7: FTIR spectra of co-A AML-ATR with formulation blend

X-ray powder diffractometry (XRPD)

To characterize the presence of amorphous nature, XRPD is considered as a gold standard method. XRPD does not detect the presence of amorphous form per say but instead detects the absence of crystallinity in the samples. The absence of crystallinity can be confirmed by spotting the halo pattern in the diffractogram. Fig. 8, 9, and 10, shows the XRPD patterns of crystalline AML, crystalline ATR and precipitated binary amorphous mixture co-A of AML-ATR respectively. Crystalline AML and ATR samples show a number of peaks in diffractogram, which confirms the crystalline nature of the individual drug. For the precipitated amorphous samples, the XRPD patterns show the typical halo (absence of crystalline peaks) suggesting amorphousness.

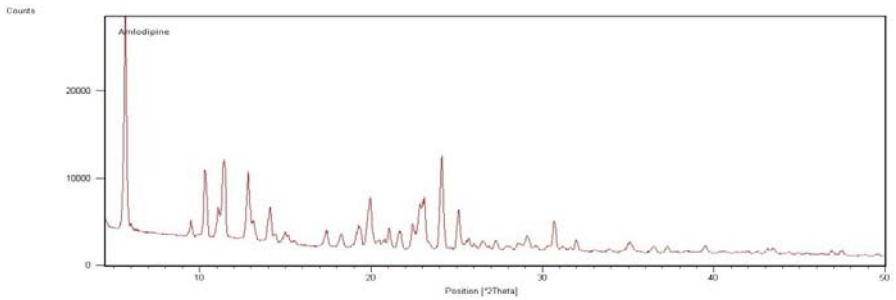

Fig. 8: X-ray powder diffractometer of AML

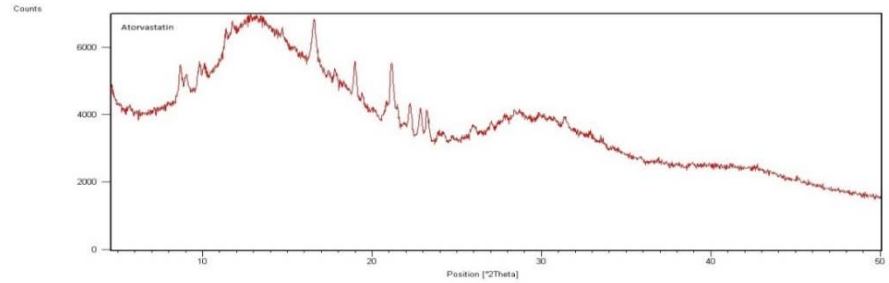

Fig. 9: X-ray powder diffractometer of ATR

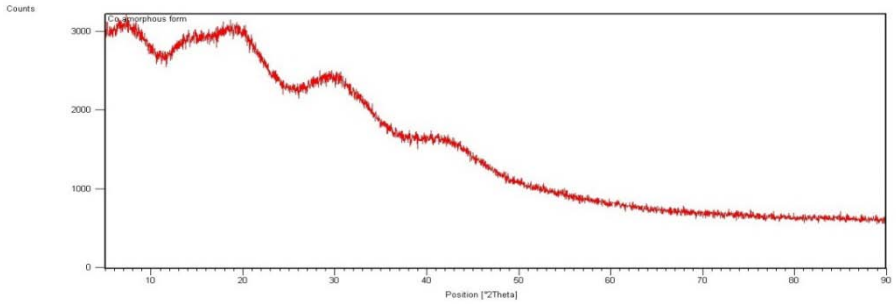

Fig. 10: X-ray powder diffractometer of co-amorphous AML-ATR

Evaluation of pre-compression parameters of formulation blend

Pre-compression parameters were performed in order evaluate the power flow properties like bulk density, tapped density, hausner's ratio, carr's index and angle of repose (table 5). Bulk density and tapped density of formulation blend F1 to F12 was performed and was found to vary from 0.276 gm/cm^3 to 0.3184 gm/cm^3 and 0.3 gm/cm^3 to 0.366 gm/cm^3 respectively. Formulation blend of F1 to F12 was introduced to hausner's ratio analysis. The values are ranges from 1.04 to 1.24 which falls under excellent, good and fair to flow properties as per the specifications. The results of carr's index or compressibility index (%) for the formulation blend F1 to F12 ranged from 4.638 % to 24.59 % that indicated excellent, good, fair, and passable flow properties.

The data obtained from the angle of repose for all the formulations were found to be in the ranges from 25.10±0.9271 ° to 28.17±0.3383 °. By looking at the ranges of pre-compression parameters, all the formulations F1 to F12 had excellent to pair to flow properties. The results of pre-formulation parameters of all formulations were in the acceptable range as per the specifications [6, 7, 23]. Hence, we were preceded by further studies.

Table 5: Evaluation of pre-compression parameters of formulation F1-F12

Formulations	Bulk density* (gm/cc)	Tapped density* (gm/cc)	Hausner's ratio*	Compressability Index* (%)	Angle of repose* Θ
F1	0.29±0.324	0.3±0.623	1.04±0.152	13.14±0.047	25.10±0.927
F2	0.3±0.214	0.31±0.236	1.05±0.702	5.4±0.154	26.60±0.34
F3	0.3±0.352	0.32±0.321	1.08±0.321	6.94±0.137	25.69±0.891
F4	0.3±0.132	0.32±0.184	1.07±0.435	6.93±0.29	26.43±0.407
F5	0.29±0.562	0.31±0.126	1.04±0.624	4.64±0.186	28.17±0.338
F6	0.32±0.142	0.34±0.161	1.08±0.2	7.49±0.347	25.76±0.576
F7	0.27±0.823	0.31±0.726	1.15±0.55	13.14±0.42	26.22±0.695
F8	0.3±0.322	0.34±0.425	1.17±0.115	13.75±0.25	26.53±0.223
F9	0.3±0.164	0.34±0.132	1.16±0.416	15.25±0.132	27.70±0.48
F10	0.3±0.527	0.37±0.121	1.10±0.802	24.59±0.292	27.54±0.388
F11	0.3±0.211	0.36±0.636	1.19±0.655	16.01±0.454	26.17±0.083
F12	0.29±0.623	3.65±0.493	1.24±0.702	19.64±0.385	25.59±0.652

*mean±SD (n=3)

Evaluation of post-compression parameters

Formulations F1 to F12 continued for the evaluation of post-compression parameters like thickness, hardness, friability and weight variation. Results were ranges from 2.6±0.01 to 2.784±0.02, 5.42±0.12 to 6.82±0.16 kg/cm², 0.10 % to 0.15 % and+1.19 % to+2.66 % and–1.039 % to-2.3174 % (table 6, 7, 8 and 9) respectively. Uniformity of drug content in the each tablet plays a very important role, if low or high drug concentration; it leads to subtherapeutic and toxic levels respectively.

Hence in order to confirm the drug concentrations in each tablet; drug content uniformity test was performed, and results ranged from 96.83±0.8% to 103.9±0.5% for AML and 98.39±0.46% to 104.5±0.6% for ATR (given in table 6, 7 and 8). Hence, these results prove that both drugs are within the limits as per the IP.

Table 6: Evaluation of post-compression parameters of formulation F1-F12

Tests / Formulations	Thickness* in (mm)	Hardness* in (kg/cm²)	Friability* in (%)	Assay*	
				Amlodipine (%)	Atorvastatin (%)
F1	2.7±0.01	5.64±0.13	0.13±0.02	101.45±0.3	98.93±0.7
F2	2.6±0.01	5.8±0.14	0.11±0.01	103.9±0.5	99.60±0.5
F3	2.7±0.01	6.42±0.15	0.14±0.01	103.4±0.5	99.73±0.3
F4	2.7±0.01	5.42±0.15	0.11±0.01	100.86±0.5	102.55±0.6
F5	2.8±0.01	6.82±0.16	0.15±0.01	102.24±0.7	104.5±0.6
F6	2.8±0.02	5.53±0.15	0.12±0.02	99.01±0.26	102.2±0.45
F7	2.7±0.01	5.97±0.17	0.10±0.01	96.83±0.8	103.03±0.4
F8	2.8±0.01	5.55±0.23	0.13±0.02	103.13±0.7	101.1±0.4
F9	2.8±0.01	5.95±0.13	0.11±0.02	100.2±0.53	98.39±0.46
F10	2.8±0.01	5.62±0.21	0.12±0.01	100.9±0.6	100.83±0.7
F11	2.8±0.01	5.93±0.15	0.10±0.01	100.8±0.56	99.67±0.74
F12	2.7±0.01	5.42±0.12	0.12±0.01	100.66±0.9	101.17±0.8

*mean±SD (n=3)

Table 7: Weight variation of formulations (F1-F12)

Formulation	Average weight in mg	Positive deviation (%)	Negative deviation (%)
F1	200.45	+2.2698	-1.22
F2	201.65	+1.66	-2.3059
F3	201.6	+1.19	-1.289
F4	200.65	+2.66	-2.3174
F5	200.4	+1.796	-1.696
F6	201.6	+1.686	-1.2896
F7	202.1	+1.4349	-1.039
F8	201.15	+1.91	-1.5659
F9	201.35	+2.3	-1.66
F10	201.55	+1.2155	-1.76
F11	201.4	+1.787	-2.184
F12	200.8	+2.589	-2.39

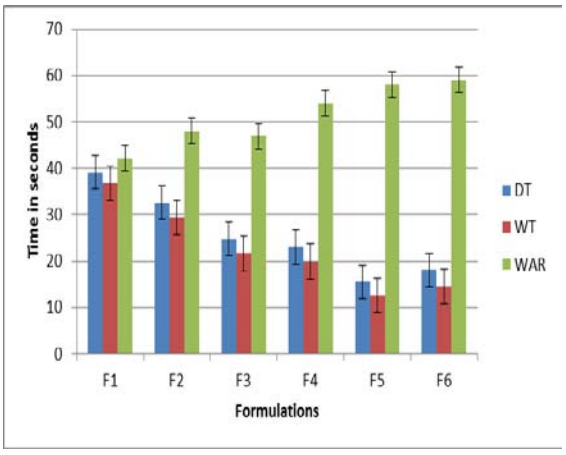

Fig. 11: Evaluations for disintegration time, wetting time, and water absorption ratio representation in column chart from formulations F1-F6

Evaluations for disintegration time, wetting time, and water absorption ratio

Average disintegration times of formulations F1 to F12 ranges from 15.53±0.5507 to 39.16±0.2886 sec and it was observed the decrease in disintegration time with an increase in concentrations of super-disintegrants [i.e. sodium starch glycolate (SSG) and crospovidone] hence, disintegration time is inversely proportional to super-disintegrant concentrations.

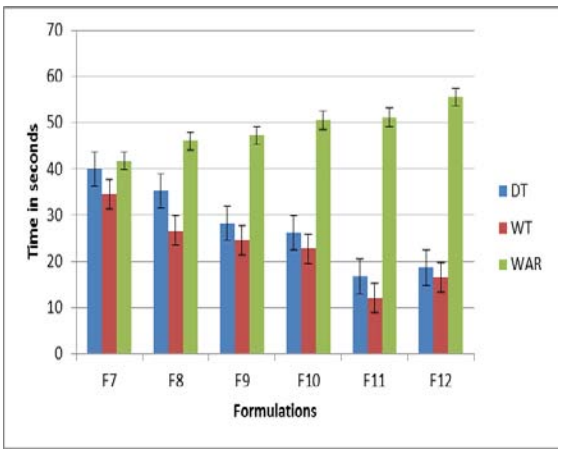

Fig. 12: Evaluations for disintegration time, wetting time, and water absorption ratio representation in column chart from formulations F7-F12

The formulation F2, F4, F8, and F10 containing crospovidone as a super-disintegrant has lesser disintegration time compared to the SSG containing formulation F1, F3, F7, and F9. But in the formulations, F5, F6, F11 and F12 visa-verse effects has been observed with respect to SSG and crospovidone. In wetting time, similar effects have been observed as seen in the disintegration time. And it also observed that, wetting time of all the formulations is lesser than that of the disintegration time and which is ranges from 12.06±0.208 to 36.73±0.3055 sec. In water absorption ratio, an increase in concentrations of super-disintegrants also increases the water absorption ratio (i.e. directly proportional) and which is range from 41.7±0.8885% to 59.03±0.2516%. The results are in the specified limits as per IP and are given in fig. 11 and 12

In vitro dissolution studies

In vitro drug release of both AML and ATR from the formulation F1 to F6 were increases gradually due to the amorphous nature and effect of super-disintegrants and at 30th min % cumulative drug release (CDR) of F1-67.27% (AML) and 68.06% (ATR), F2-71.96% (AML) and 76% (ATR), F3-76.16% (AML) and 80% (ATR), F4-78.23% (AML) and 82.03% (ATR), F5-101.7% (AML) and103.9% (ATR) and F6-96.03% (AML) and 97.66% (ATR). Similar effects observed in the formulations F7 to F12 i.e. increase in % CDR with increase in concentration super-disintegrants in formulations of F7-60.16% (AML) and 48.36% (ATR), F8-62.93% (AML) and 51.63% (ATR), F9-69.06% (AML) and 61.23% (ATR), F10-76.83% (AML) and 64.6% (ATR), F11-88.83% (AML) and 86.73% (ATR) and F12-87.16% (AML) and 84.63% (ATR). But the %CDR of the formulation F1 to F6 containing Co-A AML-ATR is much higher than that of the formulation F7 to F12 containing crystalline ATR and AML counterpart, which is mainly, corresponds to the amorphous nature of both AML and ATR. Among twelve formulations F5, F6, F11 and F12 are satisfying the IP specification limits (fig. 14, 15, 16 and 17). Results from all above-mentioned pre-formulation, pre-compression, and post-compression parameters concluded that F5 and F6 are considered as best and optimized formulations among all.

Stability study

Optimized formulations F5 and F6 were subjected to stability studies at 25 °C±2 °C/60%±5% RH and 40 °C±2 °C/75%±5% RH to assess their accelerated stability as per ICH guidelines Q1C for a period of 3 mo. At the end of 30, 60, and 90 d samples were evaluated.

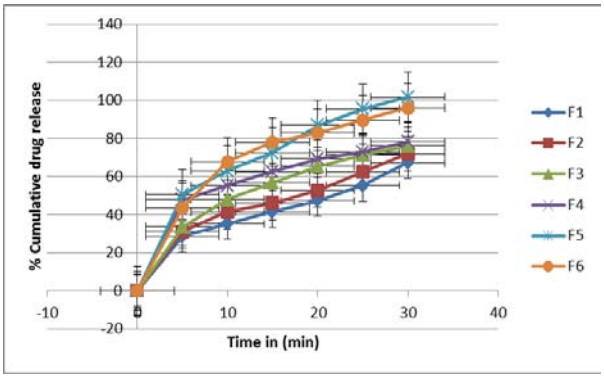

Fig. 13: *In vitro* dissolution profile of AML formulation from F1 to F6 in scatter chart

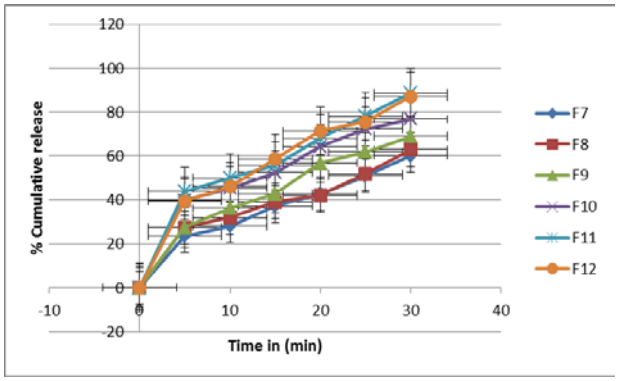

Fig. 14: *In vitro* dissolution profile of AML formulation from F7 to F12 in scatter chart

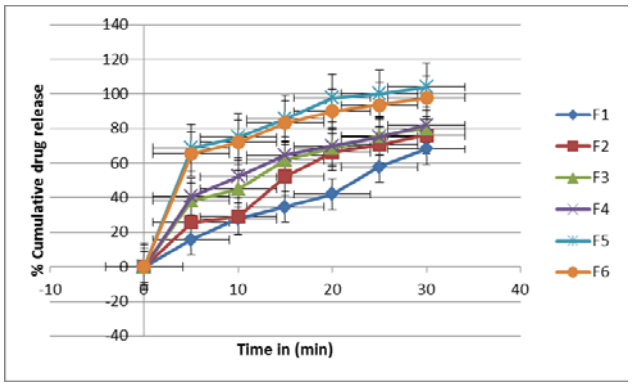

Fig. 15: *In vitro* dissolution profile of ATR formulation from F1 to F6 in scatter chart

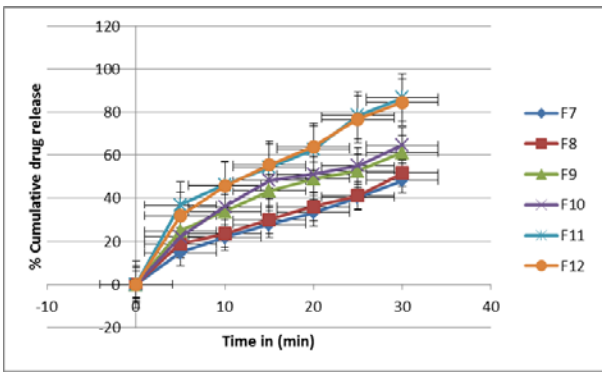

Fig. 16: *In vitro* dissolution profile of ATR formulation from F7 to F12 in scatter chart

There were no major changes in the evaluated parameters like hardness, drug content, and *in vitro* dissolution pattern.

1. Stability study of optimized formulation F5 at 25 °C/60% RH:

Table 8: Evaluation of hardness and content uniformity of formulation F5

Tests		Initial	1st month	2nd months	3rd months
Hardness* in (kg/cm^2)		6.82±0.2	6.82±0.6	6.8±0.8	6.8±0.3
Drug content*	AML	102.24±0.7	101.05±0.6	99.71±0.5	98.45±0.4
in (%)	ATR	104.5±0.6	103.64±0.3	102.13±0.7	101.53±0.8

*mean±SD (n=3)

Table 9: *In vitro* dissolution profile of AML of formulation F5

Dissolution studies				
Time in (min)	**% Cumulative drug release* in (%)**			
	Initial	**1st month**	**2nd months**	**3rd months**
0	0.0	0.0	0.0	0.0
5	50.63±0.6027	48.56±0.5876	47.36±0.5789	47.04±0.1278
10	63.03±0.1527	61.02±0.9823	59.57±0.6742	58.56±0.2598
15	72.4±0.45825	70.54±0.6428	69.45±0.6872	68.75±0.4872
20	86.9±0.81853	85.98±0.578	82.75±0.6428	81.26±0.3245
25	95.56±0.3511	94.67±0.6248	93.87±0.78521	90.87±0.6548
30	101.7±0.6244	100.01±0.5784	98.58±0.5741	97.25±0.784

*mean±SD (n=3)

Table 10: *In vitro* dissolution profile of ATR of formulation F5

Dissolution studies				
Time in (min)	**% Cumulative drug release* in (%)**			
	Initial	**1st month**	**2nd months**	**3rd months**
0	0.0	0.0	0.0	0.0
5	68.66±0.585	67.54±0.3256	66.54±0.8726	64.68±0.8753
10	74.93±0.2081	72.64±0.5873	70.64±0.5426	68.95±0.2645
15	85.56±0.550	82.45±0.8246	81.02±0.6874	79.31±0.8562
20	97.53±0.6110	95.83±0.5721	92.97±0.4875	90.15±0.3569
25	100.13±0.152	99.87±0.8254	98.56±0.816	97.64±0.2593
30	103.9±0.6557	101.67±0.3561	100.96±0.2546	99.87±0.2563

*mean±SD (n=3)

2. Stability study of optimized formulation F6 at 25 °C/60% RH:

Table 11: Evaluation of hardness and content uniformity of formulation F6

Tests		**Initial**	**1st month**	**2nd months**	**3rd months**
Hardness* in (kg/cm^2)		5.53±0.1	5.52±0.5	5.48±0.6	5.35±0.8
Drug content* in (%)	AML	99.00±0.2	98.17±0.2	97.21±0.6	96.26±0.3
	ATR	102.2±0.4	101.06±0.9	100.8±0.8	99.19±0.4

*mean±SD (n=3)

Table 12: *In vitro* dissolution profile of AML of formulation F6

Dissolution studies				
Time in (min)	**% Cumulative drug release* in (%)**			
	Initial	**1st month**	**2nd months**	**3rd months**
0	0.0	0.0	0.0	0.0
5	43.7±0.6244	43.12±0.5463	40.57±0.2651	38.02±0.8754
10	67.76±0.7098	66.7±0.3542	64.89±0.2671	63.87±0.1257
15	78.03±0.1523	76.36±0.2354	75.12±0.3215	74.68±0.1273
20	82.93±0.7767	80.58±0.1686	78.35±0.8751	76.54±0.2151
25	89.63±0.7098	87.98±0.1257	86.12±0.8756	84.98±0.1248
30	96.03±0.1523	95.64±0.8792	94.16±0.2354	91.87±0.8793

*mean±SD (n=3)

Table 13: *In vitro* dissolution profile of ATR of formulation F6

Dissolution studies				
Time in (min)	**% Cumulative drug release* in (%)**			
	Initial	**1st month**	**2nd months**	**3rd months**
0	0.0	0.0	0.0	0.0
5	65.23±0.5859	62.26±0.8742	60.85±0.1283	58.67±0.2641
10	71.96±0.1527	70.65±0.1274	68.93±0.364	66.82±0.1279
15	83.16±0.3785	81.92±0.4785	79.34±0.521	78.36±0.8462
20	89.8±0.7211	88.56±0.8753	86.24±0.2682	84.67±0.4582
25	93.53±0.5507	91.98±0.1249	90.23±0.1248	89.82±0.8754
30	97.66±0.4725	96.21±0.1934	94.87±0.2642	91.98±0.1125

*mean±SD (n=3)

3. Stability study of optimized formulation F5 at 40 °C/75% RH:

Table 14: Evaluation of hardness and content uniformity of formulation F5

Tests		Initial	1st month	2nd months	3rd months
Hardness* in (kg/cm²)		6.82±0.2	6.79±0.3	6.60±0.2	6.51±0.3
Drug content* in (%)	AML	102.24±0.7	100.14±0.3	98.16±0.5	97.01±0.5
	ATR	104.5±0.6	103.2±0.1	102.03±0.4	99.69±0.3

*mean±SD (n=3)

Table 15: *In vitro* dissolution profile of AML of formulation F5

Dissolution studies				
Time in (min)	% Cumulative drug release* in (%)			
	Initial	1st month	2nd months	3rd months
0	0.0	0.0	0.0	0.0
5	50.63±0.6027	46.21±0.5428	46.12±0.7937	45.98±0.8274
10	63.03±0.1527	60.12±0.8326	57.04±0.1272	57.1±0.3462
15	72.4±0.45825	69.81±0.1243	68.03±0.1426	66.83±0.1274
20	86.9±0.81853	83.15±0.6378	80.14±0.3417	78.90±0.4783
25	95.56±0.3511	93.01±0.8172	92.12±0.1281	88.63±0.1932
30	101.7±0.6244	98.91±0.3762	97.03±0.8264	96.03±0.2631

*mean±SD (n=3)

Table 16: *In vitro* dissolution profile of ATR of formulation F5

Dissolution studies				
Time in (min)	% Cumulative drug release* in (%)			
	Initial	1st month	2nd months	3rd months
0	0.0	0.0	0.0	0.0
5	68.66±0.585	66.83±0.7832	64.96±0.763	63.92±0.4875
10	74.93±0.2081	71.93±0.1211	68.01±0.7314	67.98±0.1765
15	85.56±0.550	80.83±0.762	80.83±0.2174	78.04±0.731
20	97.53±0.6110	93.35±0.1823	91.74±0.4231	89.98±0.875
25	100.13±0.152	98.12±0.4381	97.43±0.1238	96.79±0.6751
30	103.9±0.6557	100.28±0.8234	99.01±0.7124	97.94±0.1261

*mean±SD (n=3)

4. Stability study of optimized formulation F6 at 40 °C/75% RH

Table 17: Evaluation of hardness and content uniformity of formulation F6

Tests		Initial	1st month	2nd months	3rd months
Hardness* in (kg/cm²)		5.53±0.2	5.01±0.4	5.3±0.9	5.27±0.7
Drug content* in (%)	AML	99.00±0.3	97.42±0.8	96.77±0.7	98.26±0.2
	ATR	102.2±0.5	102.06±0.9	101.98±0.8	101.76±0.3

*mean±SD (n=3)

Table 18: *In vitro* dissolution profile of AML of formulation F6

Dissolution studies				
Time in (min)	% Cumulative drug release* in (%)			
	Initial	1st month	2nd months	3rd months
0	0.0	0.0	0.0	0.0
5	43.7±0.6244	42.98±0.6534	39.54±0.2633	37.01±0.9731
10	67.76±0.7098	68.98±0.1202	59.83±0.431	62.81±0.142
15	78.03±0.1523	75.98±0.1521	74.09±0.736	73.91±0.4739
20	82.93±0.7767	79.56±0.6352	77.56±0.2461	75.81±0.6537
25	89.63±0.7098	86.26±0.7354	85.15±0.5273	83.98±0.7235
30	96.03±0.1523	94.96±0.2671	93.97±0.4654	90.03±0.1162

*mean±SD (n=3)

Table 19: *In vitro* dissolution profile of ATR of formulation F6

Dissolution studies				
Time in (min)	**% Cumulative drug release* in (%)**			
	Initial	**1st month**	**2nd months**	**3rd months**
0	0.0	0.0	0.0	0.0
5	65.23±0.5859	61.75±0.7361	59.74±0.6373	57.73±0.2638
10	71.96±0.1527	69.98±0.638	67.35±0.2672	66.72±0.452
15	83.16±0.3785	80.73±0.7823	78.93±0.3234	77.35±0.7351
20	89.8±0.7211	86.76±0.2012	87.02±0.3671	83.15±0.5312
25	93.53±0.5507	90.83±0.2736	89.73±0.5361	88.24±0.253
30	97.66±0.4725	95.34±0.6421	93.89±0.3526	90.36±0.5363

*mean±SD (n=3)

CONCLUSION

Both AML and ATR (BCS class II drugs) were successfully converted to amorphous form by the use of rotary flash evaporator apparatus. The amorphous form was confirmed by detection of halo pattern in XRPD studies. The absence of intermolecular interaction between both the APIs, APIs with formulation blend, converted the co-amorphous form of AML-ATR, and co-A AML-ATR with formulation blend. Almost one fold increase in the solubility of both amorphous AML and ATR was found as compared to their respective crystalline counterparts. The study demonstrated successful utilization of both the physical and co-amorphous forms in tablet formulations by direct compression technique using SSG and CP. The study also demonstrated a significant increase in dissolution rate not only with respect to increasing in the concentration of both SSG and CP but also dependent on nature (i.e., crystalline or amorphous form) of the APIs utilized in the formulation of tablets. Optimized formulations were found no significant changes and extremely stable in tested stability conditions for three months.

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CONFLICTS OF INTERESTS

Declared none

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