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INVITRODRUG RELEASE STUDIES AND EVALUATION FOR CO-CRYSTALS OF ATAZANAVIR

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ABSTRACT

The present study is of formulation and evaluation of Atazanavir co-crystals to improve drug oral bioavailability. Atazanavir co crystals were prepared by sovent evaporation technique using Oxalic acid, succinic acid, saccharin, were as co crystal formers, various formulations were prepared these polymers. The Atazanavir co-crystals were characterized with respect to IR, DSC, XRD, angle of repose, bulk density, tapped density, Carr's index, Hausner's ratio, and stability studies and all the results indicated that the co-crystals were having good flow nature. Conclusion: By the in vitro dissolution studies it was concluded that the formulation prepared with oxalic acid in the concentration of 200mg (F4) was showing better result 98.97% drug release.

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INTRODUCTION

In the field of crystallography, the concept of a co-crystal was explored. The concept of host-guest chemical will include many categories of compounds including hydrates, solvates and cathrates. Hundreds of co-crystallisation examples are published every year.

Although a co-precise crystal's definition remains the subject of discussion, most solid state chemist chemists today accept that it can be described as "solids consisting of single-phase crystalline materials consisting of two or more molecular and/or ionic compounds in the stoychiometric ratio that are neither solvates nor simple salts."

There is also a talk about describing a pharmaceutical co-crystal, but it is simply a multi-component compound between a molecular or ionic API (Active Pharmaceutical Ingredients). Under atmospheric conditions, co-crystals are solid. Inside his book, Paul Pfeiffer split cocrystals into two categories: inorganic, organic and organic.

Natural co-crystals include organic, alkaline, alkaline, earth-salts, mineral acids and halogens including the halogenated quinones. The inorganic: Much of the herbal: organic co-crystals contained aromas, which are substantially di- or tri-nitro-aromatic. Co-crystal former is pharmacodynamically a ballast molecule (salt is also used), and the GRAS rules are valid. Yet an active molecule may also be a cocrystal former.

The API-co-crystal stoichiometric ratio in a pharmaceutical co-crystal is mostly clear (1:1, 1:2, 1:3 or vice versa). Co-critical compounds, ternary or quaternary crystals are not generally considered to be binary compounds. Co-crystals can be broken into co-crystal, co-crystal (solvates), c-ocrystals of salts (unsolvated, unhydrated or solvated, hydrated). (unsolvated, unhydrated or solvated, hydrated). Blurred and distinguishable by the position of the proton between acid and a base is the line between the salts and the co-crystals. Carboxylate proton is converted to the base hydrogen in salts, while acid carboxyle stays on the proton in co-crystals. When pKa = pKa(base) - pKa(acid) = 0 - 3, the proton transmission is unclear and the salt crystal spectrum is debated.[1]

MATERIALS

In the formation of co-crystals, the following products are used. Atazanavir, Saccharine, Succinic Acid, Methanol, Dmso, Sample for Karthikeya drugs and pharmaceutical products pvt. Ltd. Hyderabad.

METHODOLOGY

For the use of UV-Visible spectrophotometer, the analysis method had been established. In both phases, the λmax of atazanavir and calibration curve are calculated.

Drug – excipient compatibility studies

Fourier Transform Infrared (FTIR) spectroscopy:

In contrast with pure medicine, the IR spectroscopy of the physical mixtures. The resulting disc was placed in an acceptable Holder in the IRspectrophotometer Perkin Elmer and IR was measured between 3.500 and 500 cm, at a pressure of approximately 12 square metres for 3 minutes. Sampling was carefully compressed with 100 mg potassium bromide IR powder and compacted under vacuum. For any spectral shifts, the resulting spectrum was compared[4].

Differential Scanning Calorimetry (DSC) studies

Atazanavir, oxalic acid and sucinic acid were reported in thermal analyses on DSC (NETZSCH DSC 204). DSC's axis of temperature and cell constant have been historically indium calibrated. Nitrogen selection was used at a heating rate of 100C/min with a heating scale of 10°C to 130°C. Powder samples (15-30 mg) were weighed and tested as screened with the pin hole and as a guide was a vacuum aluminium pan[5].

Preparation of atazanavir co-crystals

The solvent evaporation method was used to treat atazanavir co-crystals. Atazanavir was weighed and blended in the same proportion with chosen co-crystal formers, and ample solvent was applied to the sufficient volume and allowed to evaporate at room temper. The co-crystals were then gathered and processed for further assessment.

Table 1: Composition of atazanavir co-crystals.

Formulation code	F1	F2	F3	F4	F5	F6
atazanavir	200 mg	200 mg	200 mg	200 mg	200 mg	200 mg
Methanol	1ml	1ml	-	1ml	-	1ml
DMSO	-	-	**1ml**	-	1ml	-
Succinic acid	-	-	-	-	-	200
Citric acid	-	200mg	-		200mg	-
Oxalic acid	200mg	-	-	200mg	-	-
Saccharin	-	-	200mg	-	-	-

X-Ray Diffraction studies (XRD)

Using Bruker D8 ADVANCE xrd analyser, X-Ray diffractometry experiments on co-crystals of atazanavir and atazanavir were performed. The sample holder was approximately 1.5 gms of each sample mounted on the spinning sample point. The samples were positioned horizontally, the X-ray tube and the detector were concurrently pushed along the angular range 2 to the angle. The X-Ray diffractograms obtained were compared and tested for signal shift.[6]

Evaluation of Co-crystals

Saturation Solubility studies

Excess volumes of Atazanavir and prepared water cocrystals, 0.1 N HCL in individual vials have been applied to solubility tests. At 37.0±0.5 o C for 48 h, the vials were shook. The filtering of samples by a 0.45-µ whatman philtre paper took place after 48 hours and 300nm wavelength of visibility was analysed with UV spectrophotometer. Triplicate (n=3) saturation solubility tests have been conducted [7].

Percentage yield

The functional percentage of ready crystals has been measured in order to know the percentage of efficiency or yield of the process. Co-crystals were extracted and weighted, and from the following equation the functional production (PY) was determined. Triplicate (n=3) percentage return tests were carried out[8].

Percent yield = (actual yield/theoretical yield)* 100.

Drug Content

Three samples of 10 mg have been taken and tested for the drug content for every batch of prepared co-crystals. Each sample weighed 10 mg in 100 ml flask normal and buffered a volume of up to 100 ml. A 0.45µ whathman filtres and dilutes the solutions. The solution was shifted one ml from above into a regular 10-ml container and volume up to 10 ml with 0.1N HCl. Solutions absorption at 300nm was measured. Triplicate (n=3) substance quality tests have been conducted[9].

***Invitro* release studies**

The in vitro release analysis of co-crystal preparations was performed using a 900ml 0.1N HCL USP type II dissolution method with 37±0.5 0C and stirred at 50 to 1/2 minute. For the formulation of Atazanavir and its co-crystals, dissolution experiments were carried out. After the required duration, the samples were removed, each time replaced with a fresh medium of 5mL. Via the use of a UV-Visible spectrometer (Schimadzu) with a wavelength of 300 nm, the solutions were instantly filtersed, filtered and ingested, and the volume of prescription discharged. Triplicate Invitro experiments (n=3) have been performed.[10].

Application of release rate kinetics to dissolution data:

Different models have been studied to describe opioid release kinetics. The details obtained were applied to a zero order, first-order, Higuchi and Korsmeyer-Peppas release model for the study of the mechanism of the kinetic drug release rate of the dosage type[11].

Stability studies :

Stability tests have been performed in compliance with ICH recommendations for optimal formulation. The co crystals were held for three months at 450C, 75% RH. Triplicate (n=3) stability tests were conducted[12].

RESULTS AND DISCUSSION

The goal of this analysis was to establish co-crystals of Atazanavir. Evaluations of physicochemical properties, invitro release and stability tests have all been done.

Preformulation parameters of co-crystals

Physical aspects of the powder mixture have been studied, including angle of resting, bulk mass, tapping density, car index, Hausner ratio.

Table 2.Preformulation parameters of co-crystals.

Formulation Code	Angle of Repose	Bulk density (gm/ml)	Tapped density (gm/ml)	Carr's index (%)
F1	26.01±0.12	0.49±0.35	0.57±0.27	16.21±0.35
F2	24.8±0.22	0.56±0.37	0.62±0.25	16.87±0.32
F3	22.74±0.17	0.52±0.29	0.68±0.22	17.11±0.45
F4	25.33±0.12	0.54±0.12	0.64±0.15	17.67±0.22
F5	26.24±0.29	0.53±0.25	0.67±0.12	16.92±0.37
F6	26.12±0.45	0.56±0.33	0.66±0.57	17.65±0.12

n=3, Mean±SD values

Diverse preformulation parameters were submitted to co-crystals. The repository angle suggests a strong flow property for the co-crystals. It was found to be within range 0.43 to 0.58 (gm/cm3) of bulk density of all formulations, which demonstrated that the powder has good flow proprities.The density of all formulations was found to be between 0.57 in gm/ml and 0.69 in gm/ml, suggesting the powder has good flow properties.

FTIR STUDY

FTIR spectrophotometer was used to conduct compatibility tests. The FTIR continuum of pure drug and physical drug, polymer and excipient variations was investigated. The peaks reached in drug, polymer and excipient physical mixture range were associated with drug spectrum peaks. The FTIR rankings have concluded that the peak movement in the IR drug continuum and drug mixtures, polymers and excipients does not change substantially.

Fig.1.FTIR Spectrum of pure atazanavir.

Fig.2.FTIR Spectrum of atazanavir + oxalic acid.

DSC STUDIES:

DSC atazanavir thermograms displayed a 122.5 — versus C endothermic limit corresponding to drug melting, but in thermograms of atazanavir and physical medicines and excipients no distinct endothermic summit existed, which indicated no association or complexation between atazanavir and polymers.

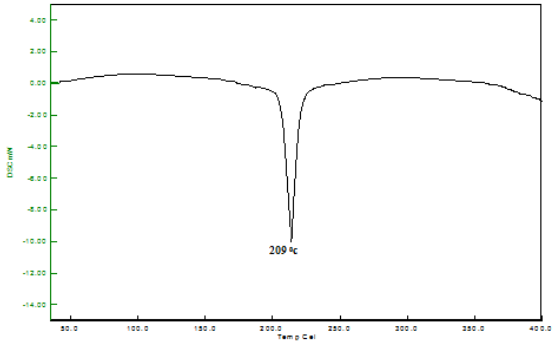


Fig 3: DSC of pure drug.

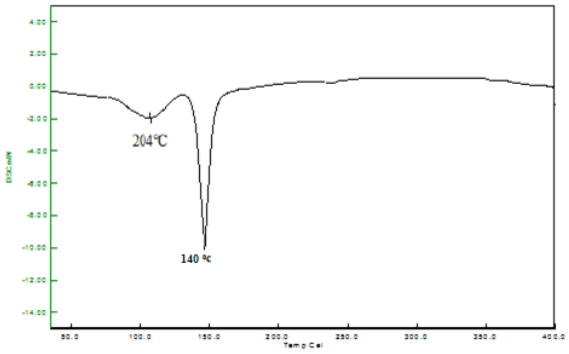


FIG 4: DSC of optimized formulation.

SEM RESULTS

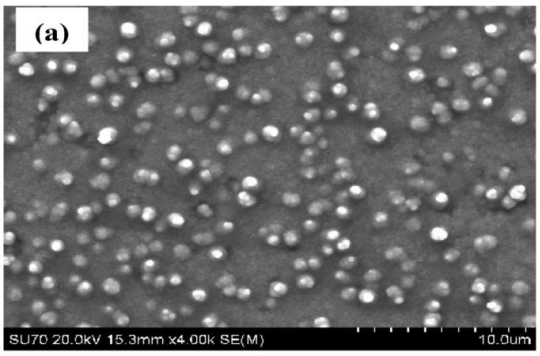


Fig 5 . SEM photograph of F4 formulation.

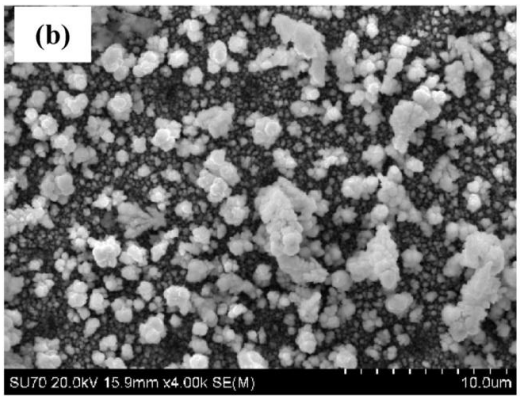


Fig 6. SEM photograph of F3 formulation.

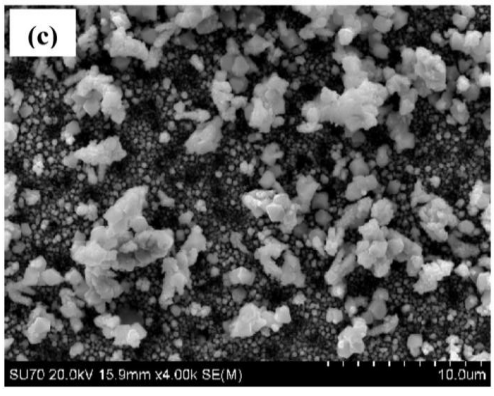


Fig 7. SEM photograph of F6 formulation.

The findings of a microscopic analysis revealed that the F4 formulated well-uniformed and crystalline crystals. While the other formulations were not standardised, it indicates that the dispersion of the medication is not uniform. The formulation F4 has therefore been refined further.

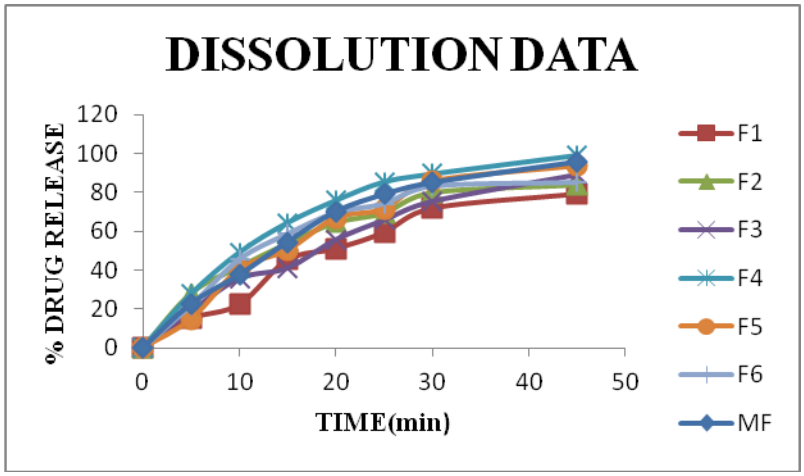
***In-Vitro* Drug Release Studies**

Both atazanavir formulations in 0.1 N HCl were performed with in-vitro release trials. The study was done over 45 minutes and the total release of drugs was measured at various times. Table 11 showed the in vitro release profiles for the formulations (F1 to F6). The accumulated drug release v/s time (h) was shown in figure8.17 respectively for F1-F3 formulation. Effects and concentrations of different ingredients have been studied in drug release. The type of polymer has been shown to affect the pattern of drug release. The rate of release decreases with the volume of polymer in the tablet formulation.

The formulas F1, F2, F3 at the end of the 45 minutes suggest a drogue release of 87.27±0.08, 88.98±0.02, 89.02±0.08%. At the end of 45minutes formulationsF4, F5, F6 showed a combined release of 98.97±0.03, 93.50±0.05, 85.11±0.02%. In contrast with other formulations that may be because of co-kristal erosion in the presence of methanol and oxalic acid combinations leading to a rise in atazanavir releases, drug release was at maximum with F4. It was then known as the optimised formulation.

Table 3: Dissolution Data of Atazanavir co-crystals.

Time (min)	F1	F2	F3	F4	F5	F6	MF
0	0	0	0	**0**	0	0	0
5	15.02±0.27	28.04±0.19	18.87±0.17	**27.73±0.12**	14.86±0.19	21.75±0.21	22.65±0.22
10	22.32±0.21	41.65±0.27	35.66±0.21	**49.04±0.14**	40.45±0.22	45.55±0.19	37.56±0.27
15	45.28±0.19	53.81±0.17	41.06±0.27	**64.33±0.19**	50.25±0.17	58.66±0.27	54.45±0.17
20	51.08±0.17	64.53±0.21	55.63±0.22	**75.84±0.13**	66.73±0.27	69.54±0.22	69.98±0.21
25	59.44±0.19	69.43±0.27	65.71±0.14	**85.32±0.15**	71.34±0.21	74.11±0.14	78.88±0.19
30	71.9±0.14	79.98±0.19	75.27±0.19	**89.44±0.21**	85.52±0.27	83.12±0.17	84.89±0.14
45	79.27±0.27	83.98±0.21	89.02±0.17	**98.97±0.22**	93.5±0.19	85.11±0.14	95.50±0.27



n=3, Mean±SD values, MF=marketed formulation.

Fig 8. Dissolution data of all the formulations prepared.

Application of Release Rate Kinetics to Dissolution Data:

Different models have been studied to describe opioid release kinetics. The details obtained were applied to a zero order, first-order, Higuchi and Korsmeyer-Peppas release model for the study of the mechanism of the kinetic drug release rate of the dosage type.

Table 4. Release Rate Kinetics to Dissolution Data.

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG (%) RELEASE	LOG (T)	LOG (%) REMAIN
0	0	0			2.000
27.73±0.12	5	2.236	1.445	0.699	1.858
49.04±0.14	10	3.162	1.618	1.000	1.768
64.33±0.19	15	3.873	1.742	1.176	1.651
75.84±0.13	20	4.472	1.824	1.301	1.522
85.32±0.15	25	5.000	1.883	1.398	1.374
89.44±0.21	30	5.477	1.947	1.477	1.060
98.97±0.22	45	6.708	1.996	1.653	0.013

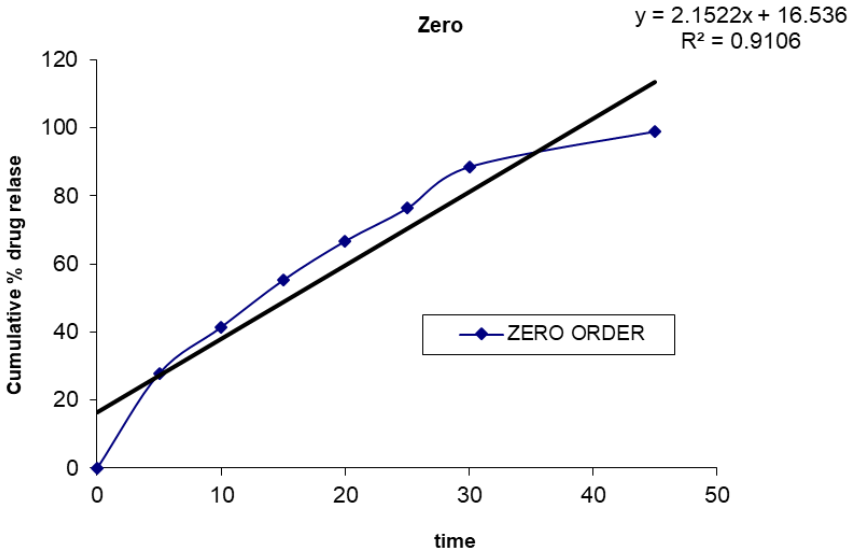


Fig. 9. Zero order plot.

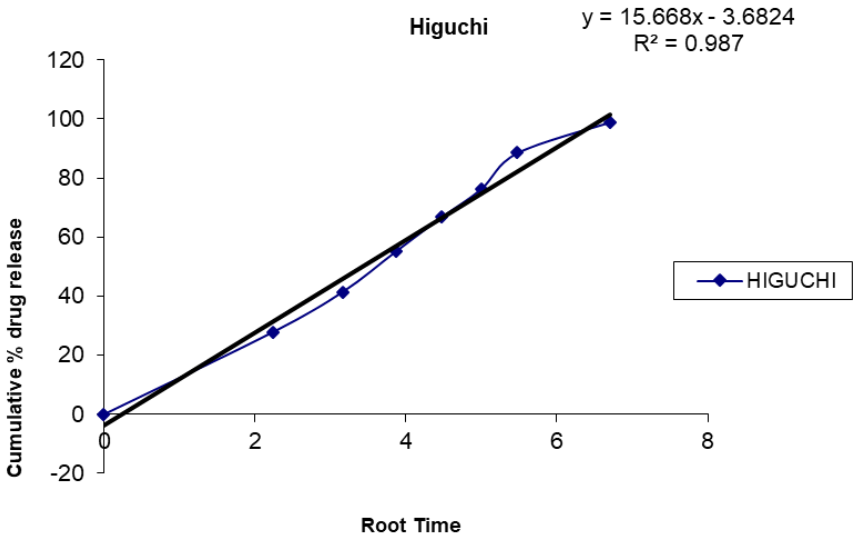


Fig.10. Higuchi plot.

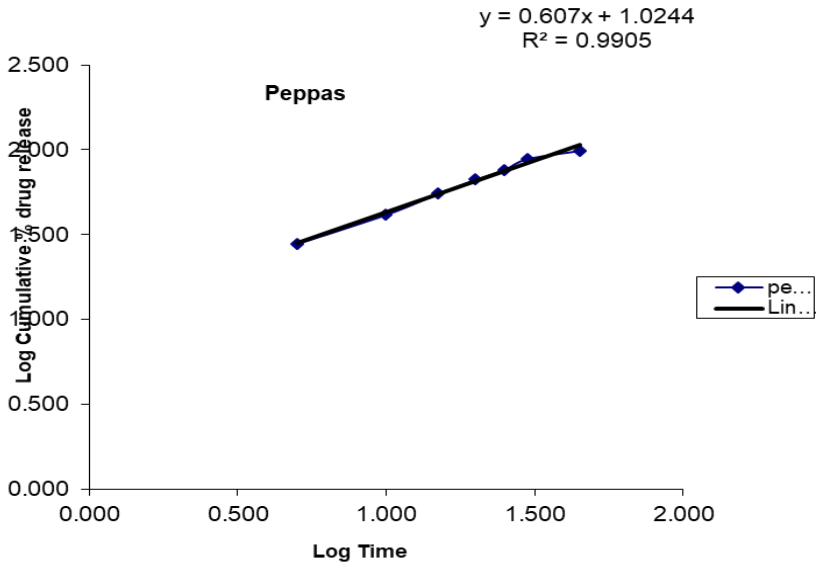


Fig. 11. Peppas plot.

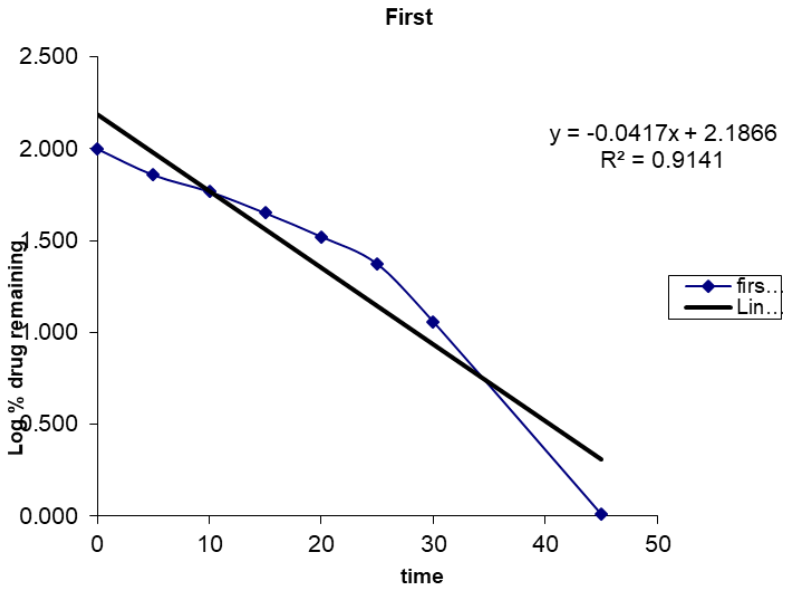


Fig. 12. First order plot.

Stability studies:

Accelerated stability tests were conducted in the streamlined formulation (F4). These experiments investigated the temperature and moisture effects of co-crystallin physical properties as a result of the optimised formulation and the release of atazanavir. 75 ± 5 percent of RH for 3 months after submitting the formulation to 45 ± 2 ± C, for different physical parameters have been evaluated. Table 13 revealed the findings. Atazanavir release at the end of the 3rd month stood at 97.75±0.09 percent. Data revealed that the optimised F4 formulation was stable because the physical parameters and in vitro disclosure of drugs did not alter substantially.

Table No:5 stability studies profile of *invitro* drug release.

S.no	Time of sampling	45^0C(75%RH)
1	1 MONTH	98.92±0.05%
2	2 MONTH	97.80±0.04%
3	3MONTH	97.75±0.09%

n=3, Mean±SD values.

CONCLUSION

The goal of this research is to formulate and evaluate co-crystals in Atazanavir with a view to growing the bioavailability of oral drugs. The solvent evaporation technology was used as co-forming solvents, using Oxalic acid, succinic acid, saccharin, and various formulations were made of these polymers. Atazanavir co-crystals were prepared. Co-crystals in Atazanavir have been characterised by the range IR,DSC,XRD, angle of repose, bulk densities, tapped mass, Carr's indice, Hausner's ratient, and stability tests.

The in vitro dissolution tests concluded that 98.97% of the drug release findings were best prepared with oxalic acid at 200 mg(F4). FTIR and DSC experiments have shown no incompatibility of the medication and polymers in order to determine the release mode. Zero order, first order, Higuchi, Korsmeyer and Peppas distribution model were submitted with the results, and the optimised formulation followed peppas release kinetics.

Recommend future Research:

Co-crystals for other antiviral drugs need to be formulated and evaluated.

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ABBREVIATIONS

FTIR - Fourier-transform infrared
DSC - Differential scanning calorimetry
XRD - X-Ray Diffraction

Conflict of interests:

None

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