Original Article

Formulation Of Tablet Of Ivermectin Co-Crystal For Enhancement Of Solubility And Other Physical Properties

Shivraj Popat Jadhav[1*], Dhananjay Machhindra Patil[2], Deepak Devidas Sonawane[3], Pratik Dilip Patil[4], Prasad Gorakshanath Ghugarkar[5], Mohammed Saad[6]

[1*,2,3] Shreeshakti Shaikshanik Sanstha's, Divine College of Pharmacy, Satana, Nashik, Maharashtra, India
[4]KBHSS Trusts Institute of Pharmacy, Malegaon, Nashik, Maharashtra, India.
[5]Dr. N. J. Paulbudhe College of Pharmacy, Ahmednagar, Maharashtra, India.
[6]Research Scholar, Govt. College of Pharmacy, Aurangabad, Maharashtra, india.

***Corresponding Author**:- Shivraj Popat Jadhav

*Shreeshakti Shaikshanik Sanstha's, Divine College of Pharmacy, Satana, Nashik, Maharashtra, India.
email id: shiva.007ind@gmail.com
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Abstract

Ivermectin is an anti-parasite medication used to treat head lice, onchocerciasis, strongyloidiasis, ascariasis, trichuriasis, and enterobiasis. Ivermectin belongs to the biopharmaceutical classification system [BCS] class II because it is having low solubility and high permeability. The present study focuses to increase the solubility of Ivermectin by use of the co-crystal technique. Co-crystals comprise a multicomponent system of active pharmaceutical ingredient [API] with a stoichiometric amount of a pharmaceutically acceptable co-former incorporated in the crystal lattice. Co-crystals of Ivermectin with different co-formers like fumaric acid, succinic acid, glutaric acid and cinnamic acid were prepared in 1:1 stochiometric ratio. Solvent assisted grinding method was used for the formation of co-crystals. Different characteristics of plain drug Ivermectin like melting point, IR, DSC, XRD, and solubility profile was compared with formed co-crystal. Among four co-crystals obtained co-crystal of Ivermectin with co-former Cinnamic acid showed an increase in solubility. Hence Ivermectin-Cinnamic acid co-crystals were further characterized. A Ivermectin-Cinnamic acid co-crystal compatibility study was performed with various excipients. Tablets were formulated by the wet granulation method and compared for in-vitro drug release studies with the marketed formulation. From the studies, it can be concluded that Ivermectin-Cinnamic acid co-crystals show higher drug release.

Keywords: Anti-parasite, Co-crystal, Hydrogen bonding, Solubility.

INTRODUCTION:

Over 50% of newly generated active pharmaceutical ingredient molecules are insoluble in water [1]. A new molecule takes roughly 10 to 15 years and millions of dollars to develop. Therefore, a variety of techniques are used to improve these compounds' solubility. Particle size reduction, solid dispersion formation, nanosuspension, co-crystal formation, salt creation, hydrotrophy, and other solubility improvement techniques are applied. [2].

Pharmaceutical co-crystals are one approach that opens up new possibilities for tackling the solubility issue. The two molecules in pharmaceutical co-crystals are arranged in a stochiometric ratio to create a new crystal structure with qualities that are frequently higher than the sum of the parts. The Active Pharmaceutical Ingredient and the co-crystal former, also known as a co-former, come together to generate the pharmaceutical co-crystals. [3].

Ivermectin [IVR] is a whitish colour non-hygroscopic, crystalline powder which is a part of Biopharmaceutical Classification System [BCS] class II. This means IVR show low solubility and high permeability. IVR is used as an anti-parasite medication used to treat head lice, onchocerciasis, strongyloidiasis, ascariasis, trichuriasis, and enterobiasis. [4] Recently it is also recommended in the treatment of COVID-19.

One the major problem associated with IVR is its less water solubility. Hence co-crystallization technique can be used to improve solubility of IVR. Presence of proton acceptor groups like-OH makes IVR a good candidate for the formation of co-crystal. In present study IVR will be screened for the formation of co-crystal. Four different co-formers like fumaric acid, succinic acid, glutaric acid and cinnamic acid will be checked for possible formation of co-crystal. These co-formers have functional groups which can form a hydrogen bond for the formation of co-crystal with IVR. Solvent-assisted grinding or solvent drop grinding is used for the formation of a co-crystal. [5] Figure 1 and 2 indicate structures of pure Ivermectin and selected co-formers.

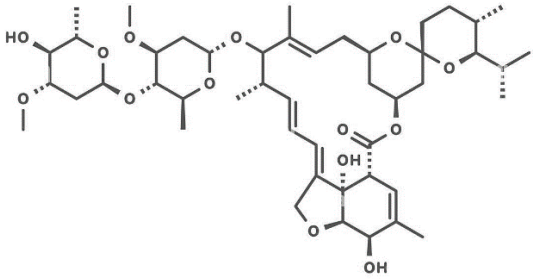

Fig. 1: Structure of IVR

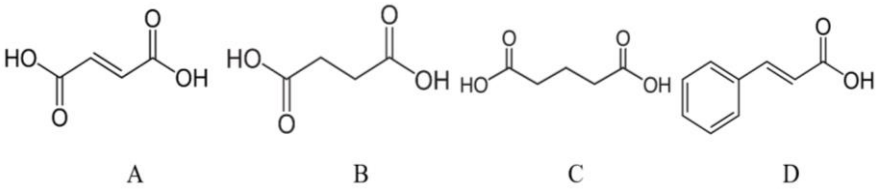

Fig. 2: Different co-formers A: Fumaric acid, B: Succinic acid, C: Glutaric acid and D: Cinnamic Acid

MATERIAL AND METHODS:

Material:

Ivermectin was obtained as a gift sample from Alkem Labs ltd., Taloja. Co-formers as well as other chemicals were purchased from Sudarshan Scientific Laboratories, Nandgaon, Nashik. Materials obtained were used without further purification or modification. Purified water USP was used where ever is required.

Method:

Preformulation studies of pure Ivermectin:

Melting point [6]: The melting point of the IVR was determined by using the capillary method. One end of the capillary tube was sealed on flame. From another open-end small amount of drug was placed at the bottom of the capillary. The capillary was then tied with a thermometer and immersed in Theil's tube containing liquid paraffin which is then heated. When the drug sample melted, the temperature was noted.

Determination of lambda max and Calibration curve: IVR was dissolved in methanol, suitably diluted with water and scanned in the range of 200 to 400 cm^{-1} spectrophotometrically on a double beam UV/Vis spectrophotometer [UV-1800, Shimadzu]. Then 2, 4, 6, 8, and 10 ppm solutions of IVR were prepared from a methanolic stock solution in water and absorbance of each solution at determined lambda max was recorded.

Solubility [7]: An excess quantity of IVR was added to water [50 ml] in stoppered glass test tubes, which were kept on a mechanical shaker at room temperature for 48 hours. The saturated solutions were filtered through Whatman filter paper, suitably diluted and the drug concentration was estimated spectrophotometrically on a double-beam UV/V spectrophotometer [UV-1800, Shimadzu].

DSC [8]: The DSC analysis of IVR was performed using the DSC instrument [Mettler]. 5 mg of IVR was accurately balanced in an aluminium pan and hermetically sealed. The sample pan and reference pan were kept in the DSC analyser. The sample was heated from ambient temperature to 300 ℃, with a heating rate of 10 ℃/minute. Pure nitrogen gas was purged at the flowing rate of 100 ml/min to maintain an inert atmosphere.

FTIR [8]: The powdered mixture of IVR and KBr was pressed in a 1:9 ratio to form a pellet. FTIR spectrum was recorded by using an FTIR spectrophotometer [Bruker] in the wavelength region of 4000 to 400 cm^{-1}.

XRD [9]: XRD of the powder sample was recorded by using PANalytical X' Pert Pro. X-Ray tube with Cu target. [Anode Material] Wavelength-1.54184 Å was used for the determination of XRD. Instrumental parameters were as 2θ angle range 10 to 90^{O}, counting time 3 s per step, counting step [2θ] 0.04^{O}.

Micromeritic properties [10, 11]: Various micromeritic properties of powder IVR like bulk density, tap density, angle of repose, Carr's index and Hausner's ratio were determined. Various formulas used for the determination of these flow properties are as follows;

Bulk density = Mass / Bulk volume
Tap density = Mass / Tap volume
The angle of repose = Height of pile / Radius of the pile
% of Carr's Index = [Tap density – Bulk density / Tap density] * 100
Hausner's ratio = Tap density / Bulk density

Preparation of Co-crystal [5]:

Co-crystals of IVR were prepared by solvent-assisted grinding method with various co-formers. IVR and co-formers were ground in the glass mortar in the ratio of 1:1 as per table 1 in presence of 2-3 drops of ethanol which later on gets evaporated.

The co-crystals formed were stored in self-sealing plastic bags in a desiccator for further studies.

Table 1: Combination of IVR and Co-former

Co-crystal code	IVR [mg]	Co-former [mg]
IVR-CA [Fumaric acid]	500	500
IVR-SA [Succinic acid]	500	500
IVR-GA [Glutaric acid]	500	500
IVR-CA [Cinnamic acid]	500	500

Evaluation of cocrystal [12]:

Formed co-crystals were evaluated for various parameters like melting point, solubility, FTIR, DSC, and XRD as per the methods discussed previously.

Co-crystal Excipient compatibility study [13]:

For the formulation of tablet dosage form, selected co-crystals as per previous studies were kept with various excipients for compatibility purpose at 40 ℃ and 75 % relative humidity [% RH] for 21 days in glass vials with and without moisture, hermetically sealed and were observed physically for, liquefaction, discoloration and gas formation as shown in table 2.

Table 2: Compatibility study of Co-crystal with excipients

Co-crystal	Excipient
IVR-CA [Cinnamic acid]	Lactose
IVR-CA [Cinnamic acid]	Starch
IVR-CA [Cinnamic acid]	Magnesium Stearate
IVR-CA [Cinnamic acid]	Avicel PH 102
IVR-CA [Cinnamic acid]	PVP K30

Formulation of the tablet [14, 15]:

Tablets of selected co-crystals [IVR-CA] and excipients were formulated by the wet granulation method. IVR-CA equivalent to the standard dose of ivermectin and excipients were weighed as per table 3.

Table 3: Formula for preparation of co-crystal tablet.

Ingredient	Use	Amount
IVR-CA	Anti-parasite	40 mg
Lactose	Filler	220 mg
Starch	Binder	20 mg
Avicel PH 102	Compression Aid	18 mg
Magnesium stearate	Lubricant/Glidant	2 mg
	Total	300 mg

Weighed excipients were passed through sieve no. 40. Co-crystal with other excipients were shifted together. A dump mass was prepared by the addition of adequate starch paste. The dump mass is then passed through sieve no. 20 and dried in an oven at 40 ℃. In the formed granules glidant was added and 300 mg tablets were compressed by use of a VBtech 8 station tablet punching machine, 10 mm punch.

Evaluation of tablet [16]:

Tablets were evaluated for various parameters like Hardness, Friability, Weight variation, Disintegration time and *in-vitro* drug release studies. The hardness of the tablet was determined by using a Monsanto hardness tester on 5 tablets. Weight variation was calculated by the use of 20 tablets with an electronic balance as per Indian Pharmacopoeia. By use of the tablet disintegration test apparatus disintegration time for 6 tablets was determined in distilled water at 37±2 ℃. Friability was determined by the use of a Roche friabilator.

Comparative dissolution studies with marketed tablets [17]:

USP dissolution test apparatus by use of the paddle method was used to study drug release of IVR-CA cocrystal and marketed ivermectin. The dissolution test was executed by using 900 ml of 0.1 N HCl at 37±0.5℃ temperature and 50 RPM paddle speed. At every 10 minutes, 5 ml sample was collected and replaced with an equal volume of new medium.

The study was carried out for 60 minutes, samples were then filtered through a 0.45 µm membrane filter and analysed by using a UV spectrophotometer [Shimadzu 1800]. The results of dissolution study of IVR-CA co-crystal tablet and marketed tablet formulation are indicated in figure 7.

RESULT AND DISCUSSION:

Preformulation studies of pure Ivermectin:

Flow properties of pure Ivermectin showed poor to passable characteristics. A saturation solubility study also indicated very slightly soluble behaviour of ivermectin in water. The results of the preformulation studies are indicated in table 4.

Table 4: Result of preformulation studies of pure Ivermectin.

Parameter	Result	Inference
Melting point	154 to 158 °C	Coincides with standard melting point 155 °C
Lambda Max	255 nm	---
Saturation Solubility	0.0035 mg/ml	---
Bulk density	0.53 gm/ml	---
Tap Density	0.69 gm/ml	---
Angle of repose	39°	Flow is fair
% Carr's index	22.99 %	Flow is passable
Hausner's ratio	1.2986	Flow is passable

Calibration curve of Ivermectin:

The calibration curve of Ivermectin was plotted in the range of 2 to 10 ppm as shown in figure 3. The coefficient of determination [R^2] 0.9942 indicated a good fit.

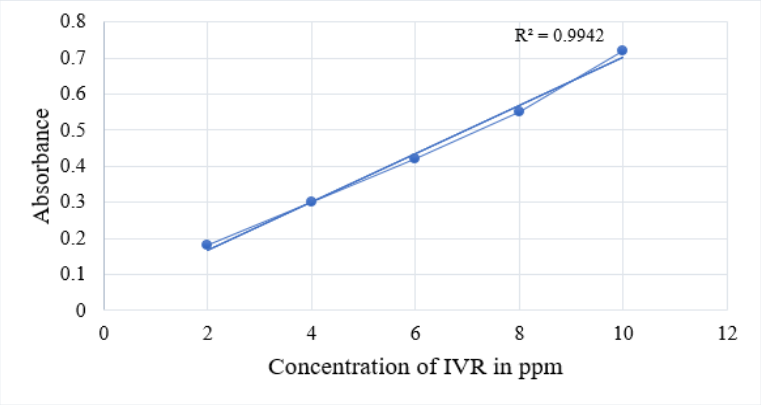

Figure 3: Calibration curve of ivermectin

DSC:

The DSC spectrum of pure ivermectin is shown in figure 4. DSC of pure IVR showed a sharp endothermic peak at 159.92 °C corresponding to the melting point of pure IVR which is 155 °C.

FTIR:

FTIR spectra of pure IVR, indicated in figure 5 shows characteristics peaks like 3481 cm^{-1}: deformation of free O–H, 2963 cm^{-1}: characteristic of methyl groups, 2933 cm-1: deformation of C–H, 1730 cm^{-1}: C=O stretching and 1181 to 1047 cm^{-1}: deformation of C–O–C.

XRD:

The XRD pattern of pure Ivermectin is shown in figure 6. The X-ray diffraction of pure ivermectin from 10 to 40 2θ values showed namouras peaks which indicates the high crystallinity of pure Ivermectin.

Evaluation of Co-crystal:

Co-crystals were evaluated based on melting point, bulk density, tap density, angle of repose, % of Carr's index, Hausner's ratio, solubility, FTIR, DSC, and XRD. Results are indicated in table 5.

Table 5: Evaluation of co-crystal on various preformulation parameters.

Parameter	IVR-FA	IVR-SA	IVR-GA	IVR-CA
Melting point (Onset)	218 °C	176 °C	158 °C	142 °C
Saturation Solubility	0.024 mg/ml	0.076 mg/ml	0.048 mg/ml	0.432 mg/ml
Bulk density	0.69 gm/ml	0.72 gm/ml	0.67 gm/ml	0.65 gm/ml
Tap Density	0.82 gm/ml	0.88 gm/ml	0.80 gm/ml	0.76 gm/ml
Angle of repose	38°	35°	36°	28°
% of Carr's index	15.11 %	17.74 %	16.41	14.54 %
Hausner's ratio	1.18	1.21	1.19	1.17

Melting points of all the formulated co-crystals were in between pure ivermectin and co-crystal former. Previous studies also shown that around 51 % of co-crystals formed are have melting point between the pure drug and co-former. The

melting point of API can be altered through the formation of a co-crystal (18). The saturation solubility of formulated co-crystals was found to be increased than pure ivermectin. Among formulated co-crystals, IVR-CA has shown high saturation solubility (0.432 mg/ml). The solubility of IVR-CA is found to be 123 times more than pure ivermectin. Also, the flow properties of most of the co-crystal were found to increase. The angle of repose of IVR-CA was found to be 28° indicating good flow characteristics. Carr's index also indicates an increase in flow properties of co-crystal (22.92 %) than pure ivermectin (18.80 %). Hausner's ratio is also found to be more for co-crystals. Overall, all the co-crystals showed superior properties over pure ivermectin. Among the prepared co-crystals, IVR-CA has shown more saturation solubility as well as other properties that are superior over other co-crystals hence IVR-CA co-crystals were selected for further studies.

DSC of IVR-CA co-crystal:

DSC spectra of IVR-CA showed a little broad peak near about 140 ℃ which is ascribed to the melting process of co-crystal as indicated in figure 4. The presence of a single endothermic peak indicates the completion of the process of crystallization.

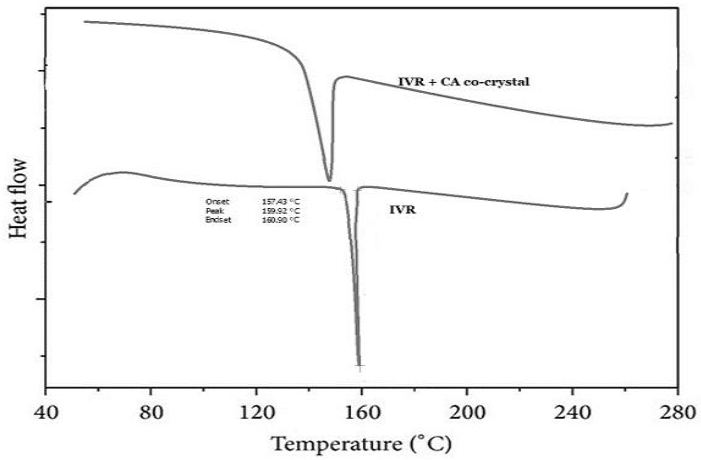

Figure 4: DSC spectra of pure IVR and IVR-CA cocrystal

FTIR of IVR-CA:

FTIR can be used as a powerful tool to study co-crystal formation. A prominent O-H stretching peak was observed in pure ivermectin spectra which is found to be vanished in IVR-CA co-crystal FTIR spectra. This can be indicative of the interaction of the cinnamic acid "-COOH" group with the "-OH" group of ivermectin as shown in figure 5. Also figure 6 indicates possible interaction between ivermectin and cinnamic acid.

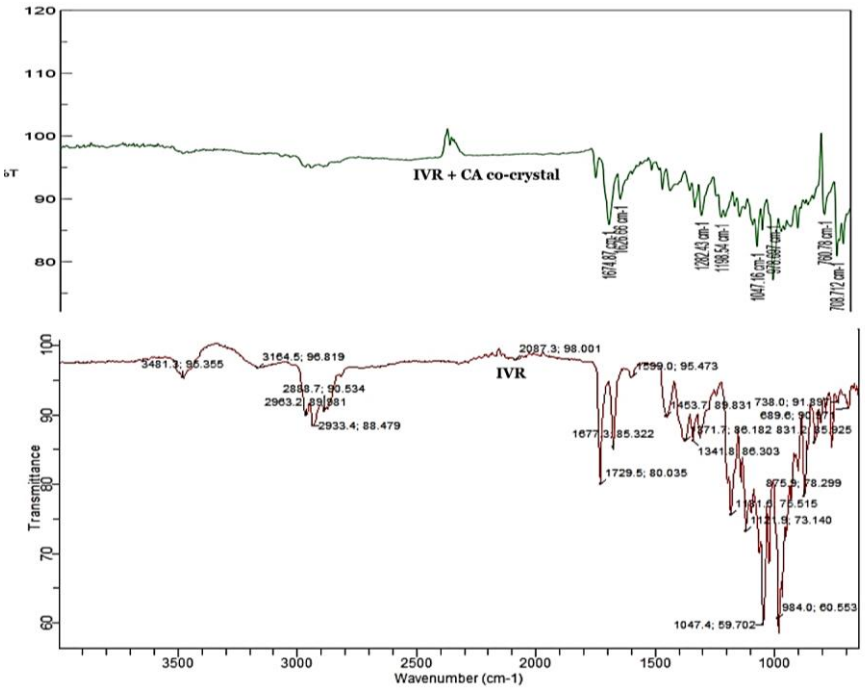

Figure 5: DSC spectra of pure IVR and IVR + CA cocrystal

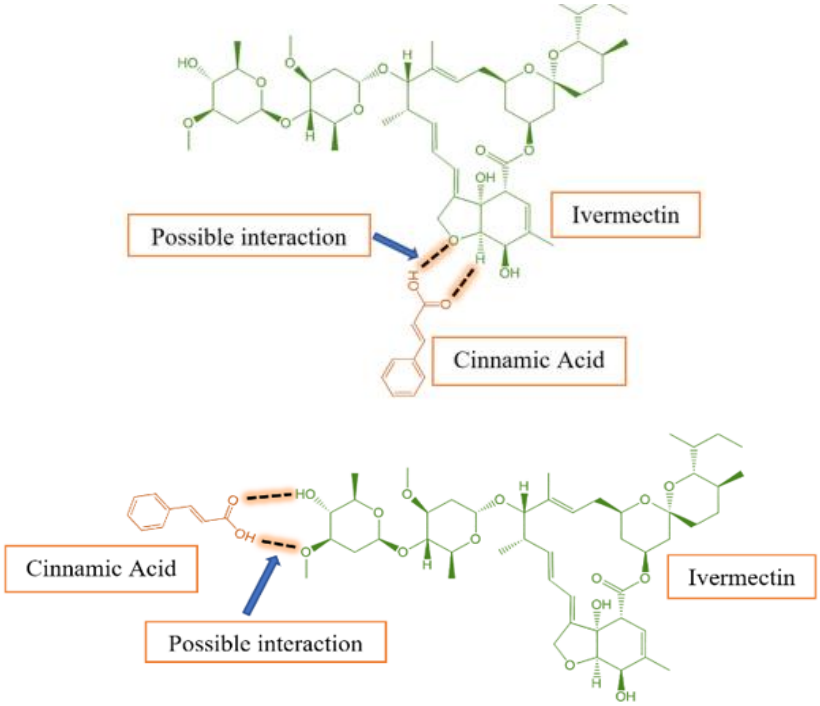

Figure 6: Possible interactions between ivermectin and cinnamic acid for formation of co-crystal

XRD of IVR-CA co-crystal:

XRD spectra of the IVR-CA co-crystal show number of peaks which confirms the crystalline structure of the co-crystal as indicated in figure 7. Newer peaks can also be observed which is indicative of the formation of a new crystalline structure.

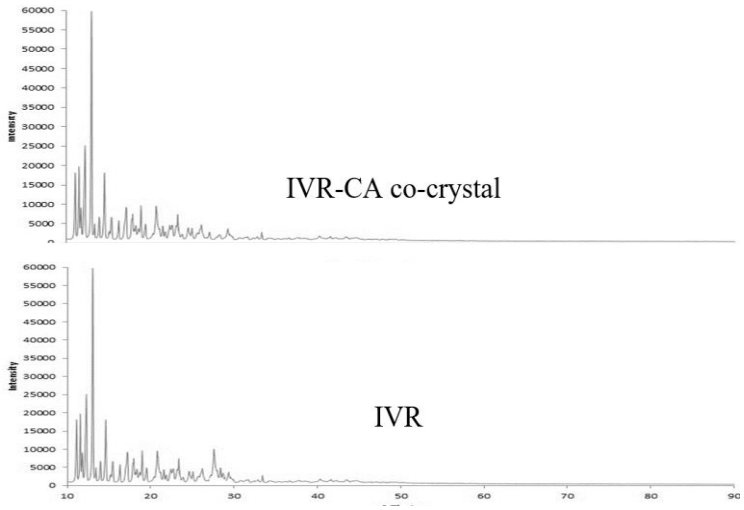

Figure 7: XRD spectra of pure IVR and IVR + CA cocrystal

Co-crystal and excipient compatibility study:

No significant change was observed during the compatibility study as shown in table 6 of co-crystals and excipients. After the compatibility study when vials were open, also there was no odd odor formation.

Table 6: Compatibility study of various excipients with co-crystals.

Co-crystal	Excipient	Liquification		Colour Change	
IVR-FA	Lactose	No change	No change	No change	No change
IVR-FA	Starch	No change	No change	No change	No change
IVR-FA	Magnesium Stearate	No change	No change	No change	No change
IVR-FA	Avicel PH 102	No change	No change	No change	No change
IVR-FA	PVP K30	No change	No change	No change	No change

Evaluation of tablets:

Tablets were assessed for parameters like hardness, weight variation, disintegration and friability. The results of the studies are indicated in table 7. All the tests were found to be passed.

Table 7: Evaluation of tablets.

Parameter	Result	Pass/Fail
Hardness	4 to 5 kg	Pass
Weight variation	Average weight of 20 tablet 305 mg, limit:320.25 to 289.75	Tests pass as per IP standards
Disintegration time	8 to 13 minutes	Pass
Friability	0.8 %	Pass

Comparative dissolution studies with the marketed formulation:

Prepared IVR-CA co-crystal tablets were evaluated with marketed tablet for drug release studies. The drug release pattern of marketed tablet and IVR-CA co-crystal tablet are indicated in figure 8. % Cumulative drug release of marketed tablet formulation after 60 minutes was found to be 56 % while % the cumulative drug release of co-crystal tablet was found to be 85 %. This clearly indicates that the dissolution profile of the drug can be modified by the use of the co-crystallization method.

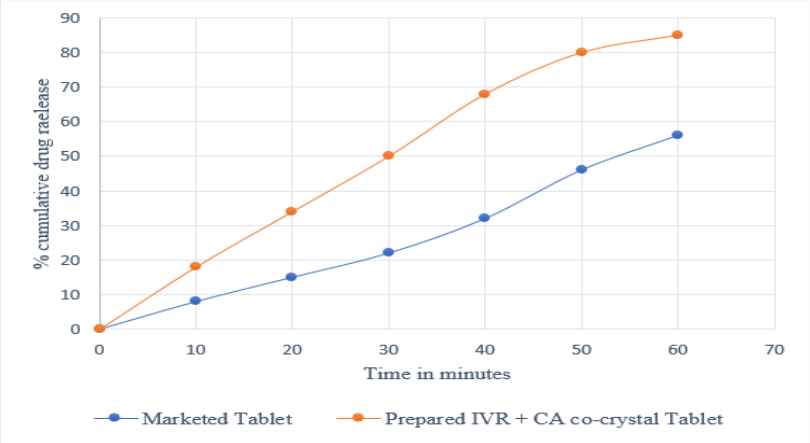

Figure 8: Comparative dissolution study of marketed tablet and formulated tablet of co-crystal

CONCLUSION:

From the conducted studies, it can be concluded that solvent-assisted grinding method co-crystal formation can be successfully utilized for enhancement of solubility and other physical properties of drugs belonging to BCS class II. Results from saturation solubility, FTIR, DSC and XRD indicate the formation of co-crystal between Ivermectin and fumaric acid. The newly formed co-crystals exhibited greater solubility and dissolution rate. This technique can be successfully employed for the solubility enhancement of other BCS class II drugs.

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