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Design and Evaluation of Oral Films Loaded with Piperine Loaded Co-Crystals

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ABSTRACT

Co-crystals are unique crystalline structures formed by at least two constituents, creating a distinctive crystalline structure with specific properties. Oral Thin Films (OTFs), also known as orodispersible films, have gained significant attention as a novel drug delivery system. Solvent evaporation is the most common method for preparing co-crystals and is typically applied for synthesizing high-quality co-crystals. The prepared oral thin films were subjected to evaluation tests like thickness, weight variation, surface pH, folding endurance, content uniformity, disintegration time, and *in-vitro* dissolution studies. The weight variation was found to be minimum. The thickness of the films was found to be uniform. Films were found to have sufficient strength. Disintegration time for the films was found within 37.5 sec.

Keywords: Co-crystals, Piperine, Oral films, disintegration time, thin films.

1. INTRODUCTION

Co-crystals are unique crystalline structures formed by at least two constituents, creating a distinctive crystalline structure with specific properties. Typically, these structures involve non-covalent interactions, including hydrogen bonds, ionic interactions, van der waals interactions, and π-interactions, which contribute to their stability and distinct characteristics. There are two main methods for preparing co-crystals: solution-based methods and solid-based methods. Solution-based methods involve the use of solvents, and the choice of solvent can influence the interactions between the components. Solid-based methods, on the other hand, involve less or no solvent and are environmentally friendly. Co-crystal engineering has found significant applications in various fields, most notably in pharmaceuticals. By altering the structure and composition of active pharmaceutical ingredients (APIs), co-crystals can improve the bioavailability and solubility of drugs. The use of co-crystals can offer a unique opportunity to modify the physicochemical properties of compounds, leading to improved therapeutic effectiveness.[1-3]

Oral Thin Films (OTFs), also known as orodispersible films, have gained significant attention as a novel drug delivery system. They were initially introduced to address issues related to swallowing difficulties in pediatric, geriatric, and other patient groups. These films disintegrate rapidly in the oral cavity, allowing for pre-gastric absorption and quick onset of action. Key components of oral thin films include the drug or active pharmaceutical ingredient (API), film-forming agents, plasticizers, surfactants, saliva simulating agents, sweetening/flavoring agents, and colorants. Each component plays a crucial role in the overall performance and patient acceptability of the film.[4,5]

Common methods for manufacturing oral thin films include solvent casting, hot-melt extrusion, semisolid casting, rolling method, and solid dispersion extrusion. Each method has its own advantages and limitations, influencing the physical properties and overall efficacy of the oral thin films. Oral thin films provide a promising alternative to traditional drug delivery systems, especially in cases where swallowing may be difficult. Their rapid disintegration and enhanced bioavailability make them a preferred option for patients across different age groups. However, challenges related to dosing, manufacturing, and stability need to be carefully considered during the formulation and development process.[6-8]

2. MATERIALS AND METHODS

2.1 Materials

Piperine, active ingredient (Yucca Enterprises), Succinic acid, co-former (S D Fine Chem Ltd.,Mumbai), Hydroxy propyl methyl cellulose (HPMC-E15), polymer (Research Lab Fine Chem Industries,Mumbai)), Propylene glycol(PG-400), plastisizer (Molychem, Mumbai), Mannitol, Sweetening Agent (S D Fine Chem Ltd., Mumbai), Citric acid, saliva stimulating agent (Molychem, Mumbai), Sodium Saccharin, sweetening Agent (S D Fine Chem Ltd., Mumbai), Ethanol, solvent (Molychem, Mumbai), phosphate buffer 6.8, and distilled water.

2.2 Methods

Spectroscopic studies

Preparation of pH 6.8 Buffer: In the preparation of 0.2M KH_2PO_4, 1.3609g of Potassium hydrogen phosphate was weighed and added to 50 ml of distilled water. Then, 0.2g of Sodium hydroxide was dissolved in 25 ml of distilled water to make 0.2M NaOH. Afterward, 22.4 ml of NaOH solution was measured and added to 50 ml of KH_2PO_4 solution, which was then made into 1000 ml by adding distilled water.

Determination of λ max:Standard stock solution was prepared by dissolving an accurately weighed quantity of Piperine in a suitable volume of 100ml of Potassium hydrogen phosphate buffer and scanned in the range 200 – 400 nm against the buffer as blank. The wavelength of maximum absorption was determined for the drug.

Preparation of 40 µg/ml Drug Stock Solution:The preparation of the 40 µg/ml drug stock solution involved weighing an appropriate amount of the pure drug compound (e.g., 1 g) and dissolving it in a sufficient amount of phosphate buffer (pH 6.8) to make the stock solution. The drug was completely dissolved through thorough mixing or sonication if necessary. Further, different concentrations i.e., 4, 8, 12, 16, 20 µg/ml solutions were prepared from the stock solution. [9-11]

Procedure for establishing the calibration curve of Piperine: A pure drug solution of 40µg/ml was prepared to attain its aliquots (4, 8, 12, 16, 20 µg/ml) which are dissolved in certain quantities of phosphate buffer pH 6.8. The absorbance of these aliquots was measured using UV Spectrophotometer at 342 nm. The results were presented in a table and graph with direct line equation was plotted and $R^2$is compared and complied with Beer Lamberts Law.[12]

2.3 Preparation of Piperine Co Crystal with Succinic Acid

Solvent evaporation is the most common method for preparing co-crystals and is typically applied for synthesizing high-quality co-crystals. In this approach, the co-crystal was prepared by taking appropriate stoichiometric ratio of piperine 28.53 mg and succinic acid 23.6 mg (1:2) ratio with a suitable solvent i.e., ethanol and triturated with the help of mortar and pestle and then evaporated the solvent to obtain the co-crystal. The PSA cocrystals were obtained and stored in a desiccator until further analysis. [13]

3. EVALUATION OF CO CRYSTALS

3.1 Solublity studies:

The solubility was determined by dissolving excess quantity of pure drug, physical mixture and co-crystals in the 100 ml conical flask containing 25 ml of water. The vials were subjected to agitation on rotary shaker and allowed to stand for equilibrations for 24 hr and 48 hr. The samples were filtered after 24 hr and 48 hr, diluted with distilled water. The absorbance of diluted samples was measured using UV spectrophotometer at 342 nm.

3.2 Drug - excipient compatibility studies:

FTIR -ATR spectra of piperine and optimized batch of co-crystals were obtained on a FTIR-ATR (Bruker alpha) using opus software. In the present work, the samples was taken in separate watch glasses and dried in an oven at 100ºC for 30 minutes, cooled and subjected to IR spectroscopy. The respective sample was placed on the crystal and kept in the path of IR radiation and scanned for 16 times. The spectra obtained were recorded which consists of absorption bands from sample and background (air and solvent).The scanning range was 600-4000cm^{-1}.

3.3 Differential scanning calorimetry:

The thermal behavior of cocrystal was determined by Differential scanning calorimetry (DSC) studies. Weighed samples were heated in aluminum pans under a nitrogen stream. The instrument was calibrated using indium and empty aluminum pan was used as a reference.[14-16]

3.4 Preparation of Oral Films

Preparation of blank film:

Polymer HPMC E15 of different mg were measured i.e., 100mg ,200 mg, 300mg 400 mg,500 mg and added in the solvent separately in a glass beaker and kept aside for soaking for half an hour. The solution was stirred for 15min to obtain a homogenous solution. With a time interval of 10-15 min, other excipients saccharin, mannitol, Propylene glycol (PG), and citric acid were added. were further added in the order. The solution was kept for stirring for approximately 1-2 hr on the magnetic stirrer. The solution was then kept aside for few minutes to remove any air bubbles entrapped. The mixture was poured into a glass petridish evenly and left for drying for 24hr or dried in a hot air oven. The accurate volume of polymer in the film was chosen.

Preparation of piperine oral thin film:

S.No.	Components	F1	F2	F3	F4	F5
1	Piperine (mg)	5	5	**5**	5	5
2	HPMC E15 (mg)	100	200	**300**	400	500
3	Sodium Saccharin(mg)	25	25	**25**	25	25
4	Mannitol (mg)	25	25	**25**	25	25
5	Propylene glycol (ml)	0.15	0.1	**0.15**	0.15	0.15
6	Citric Acid (mg)	5	5	**5**	5	5

The oral thin film was prepared with the solvent-casting technique. In a beaker required quantity of ethanol was taken, the polymer was soaked in ethanol for about an hour. The solution was agitated on a magnetic stirrer for half an hour. The drug (piperine) was dissolved in the polymeric solution and stirred for 15 minutes. Later, with an interval of 5-10min the excipients, saccharin, mannitol, Propylene glycol (PG), and citric acid were added. After one hour of mixing the polymeric solution and other excipients, a homogenous solution was obtained. Then the solution was kept aside for 30min to extract any air bubbles entrapped. The solution was then poured in a petridish and are kept for drying either for 24hrs at room temperature or put under 40-50℃ for 20min in a hot air oven. The slides were then carefully removed. For further evaluations, the films that were transparent and clear were selected and cut into $4cm^2$ (2× 2) and carefully placed in parchment paper and held in desiccators. [17-20]

Composition of different oral thin films containing piperine

3.5 Evaluation of oral thin films

Physical characterization of fast dissolving oral thin films was be carried out by visual inspection for characteristics such as colour, thickness, brittleness, transparency, surface smoothness, and tack property. The prepared oral thin films were subjected to evaluation tests like thickness, weight variation, surface pH, folding endurance, content uniformity, disintegration time, and *in-vitro* dissolution studies.

1. ***Weight variation*:** Each film was cut from the cast films in 4 cm (2x2cm sq.).Each film was weighed, calculated the average and also checked for uniformity.
2. ***Film thickness:*** The thickness of a single film was measured by a digital verniercallipers at different places.
3. ***Folding Endurance:*** Flexibility of oral thin films is important as the key is to the administration of film without breaking. A strip of 2x2cm² was subjected by folding the film repeatedly at 180° at the same place until a visible crack was observed and the values are noted.
4. **Appearance:** Each film was checked properly for its appearance, shape, and size.
5. ***Drug content uniformity:*** Films were transferred into graduated flasks containing 100ml phosphate buffer pH 6.8 and continuously stirred. The solution was filtered, suitably diluted, and analysed spectrophotometrically at 342 nm and the drug content was calculated
6. ***In-vitro Disintegration studies:*** Petri dish method was used for disintegration studies. The film was placed in the glass petri dish containing 10 ml of buffer of 6.8pH and time taken by the film to disintegrate was recorded.[20-25]

4. RESULTS AND DISCUSSION

In this study, we developed, prepared, and evaluated oral thin films containing co-crystals of Piperine-Succinic acid.

4.1 Preformulation study:

The organoleptic properties of piperine were examined and the following observations were recorded.

Table no. 1: Organoleptic properties:

S. No	Parameter	Drug
1	Color	Light yellow

2	Odour	Pungent
3	Appearance	Solid
4	Melting point	285.343 ºC
5	**Solvents**	**Solubility**
	Water	Very slightly soluble [0.04mg/mL]
	Ethanol	Soluble [57mg/mL]

Spectroscopic Studies

***Determination of λ max*:** The λ max of **Piperine** was found to be **342nm** using UV Spectrophotometer.

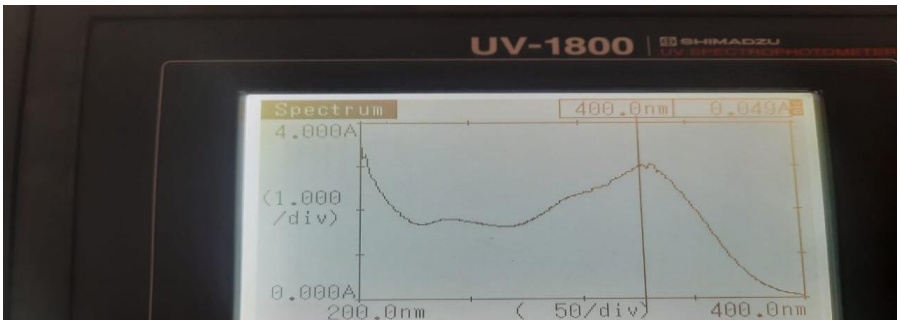

Fig 1: UV absorption spectra of Piperine.

Calibration curve of Piperine

Standard stock solution: The standard stock solution was prepared by dissolving 4mg of the drug in 100ml of phosphate buffer 6.8.

pH 6.8 Calibration curve of Piperine in phosphate buffer: Absorbance was taken at 342 nm for the above solution to attain 4, 8, 12, 16 20 µg/ml dilutions. Absorbance for these concentrations was taken and the linearity plot at 342 nm with absorbance is shown in following table-

Table no. 2: Calibration curve data of Piperine in pH 6.8 phosphate buffer

S.no	Concentration (µg/ml)	Absorbance
1	0	0
2	4	0.12
3	8	0.27
4	12	0.37
5	16	0.46
6	20	0.57

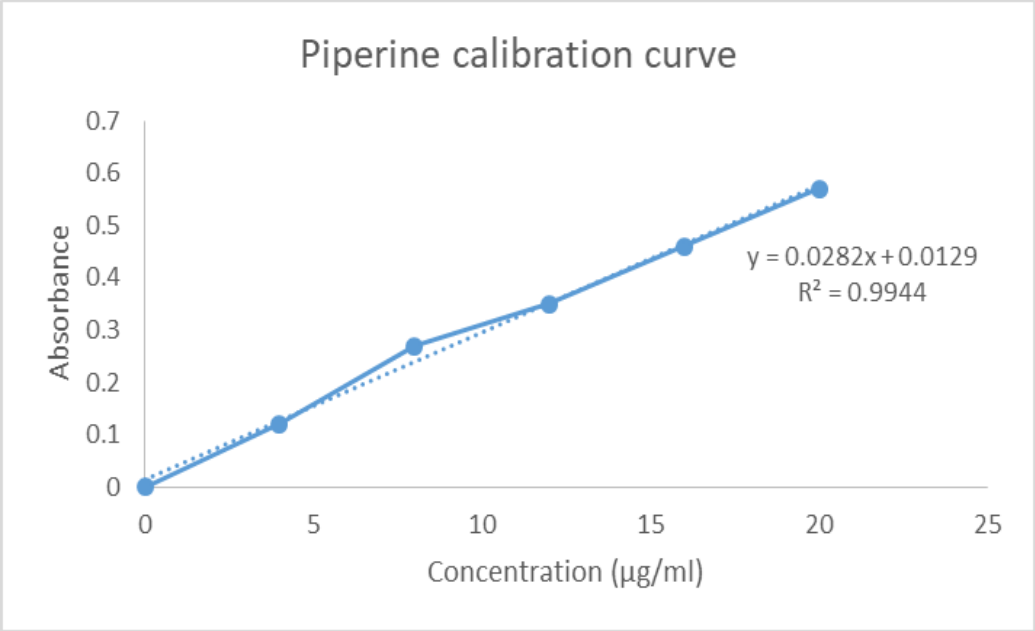

Fig 2 : Calibration curve of Piperine

***Solubility studies*:** The solubility of piperine - succinic acid co-crystal was found to be greater than pure drug.

***Drug - excipient compatibility studies*:** The functional groups found in pure drug i.e., piperine and the conformer succinic acid were retained in the co-crystal prepared. However, no strong new peaks were not observed. This says that there is no interaction between the compounds and they are compatible.

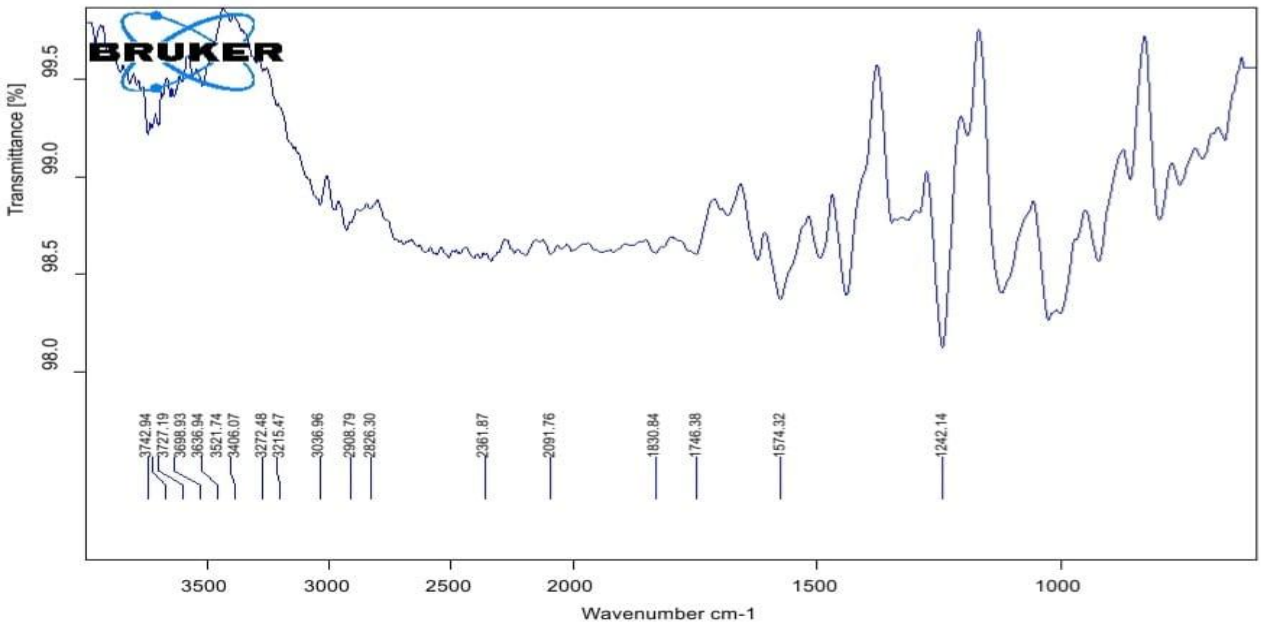

Fig 3: FT - IR of Piperine.

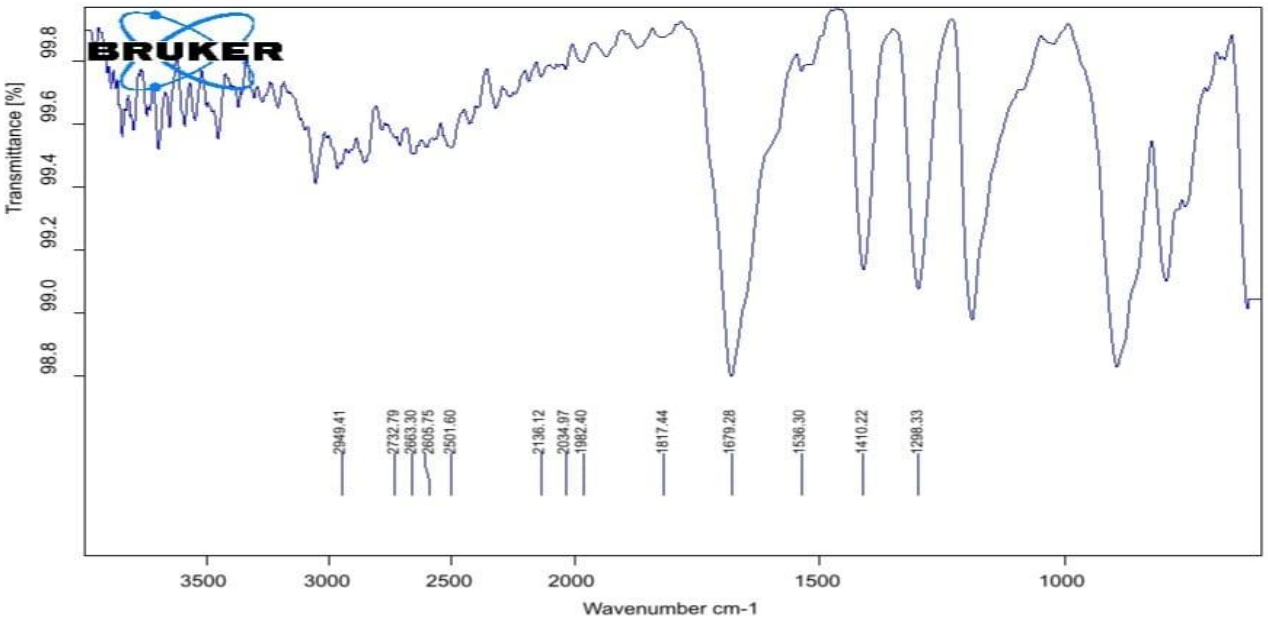

Fig 4: FT-IR of succinic acid

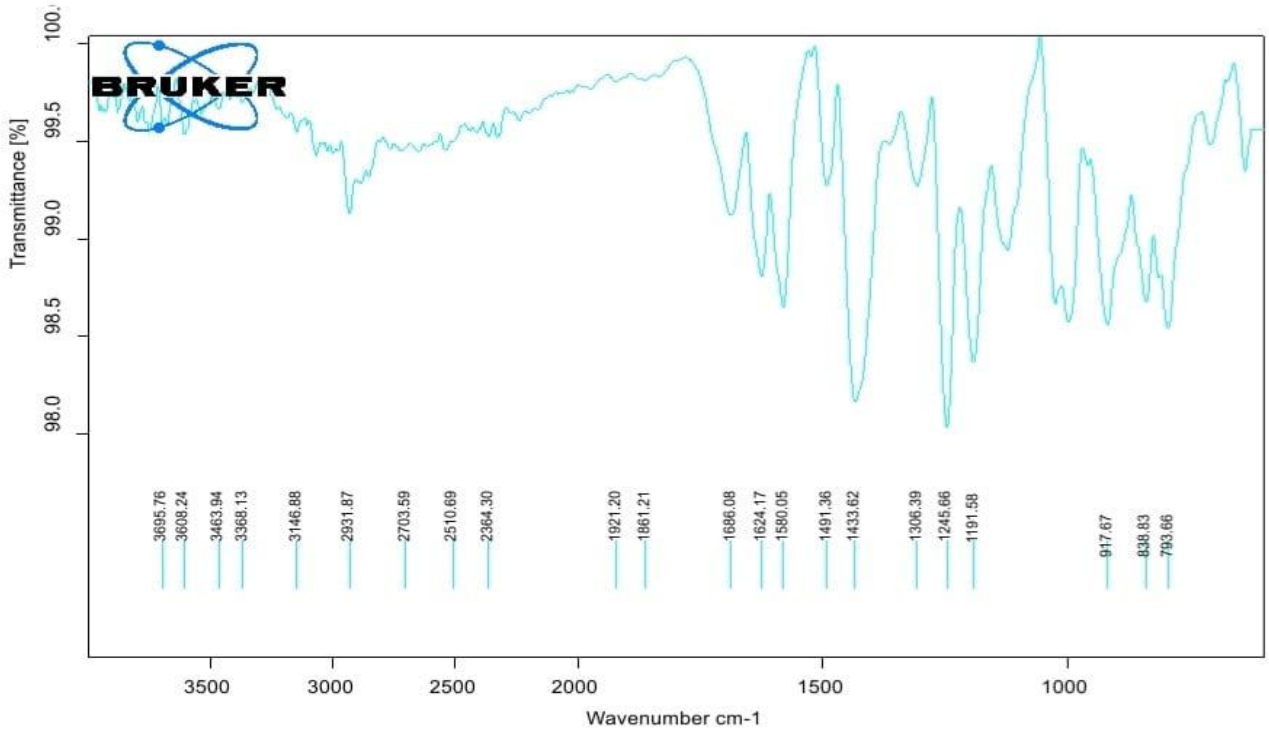

Fig 5: FT-IR of physical mixture

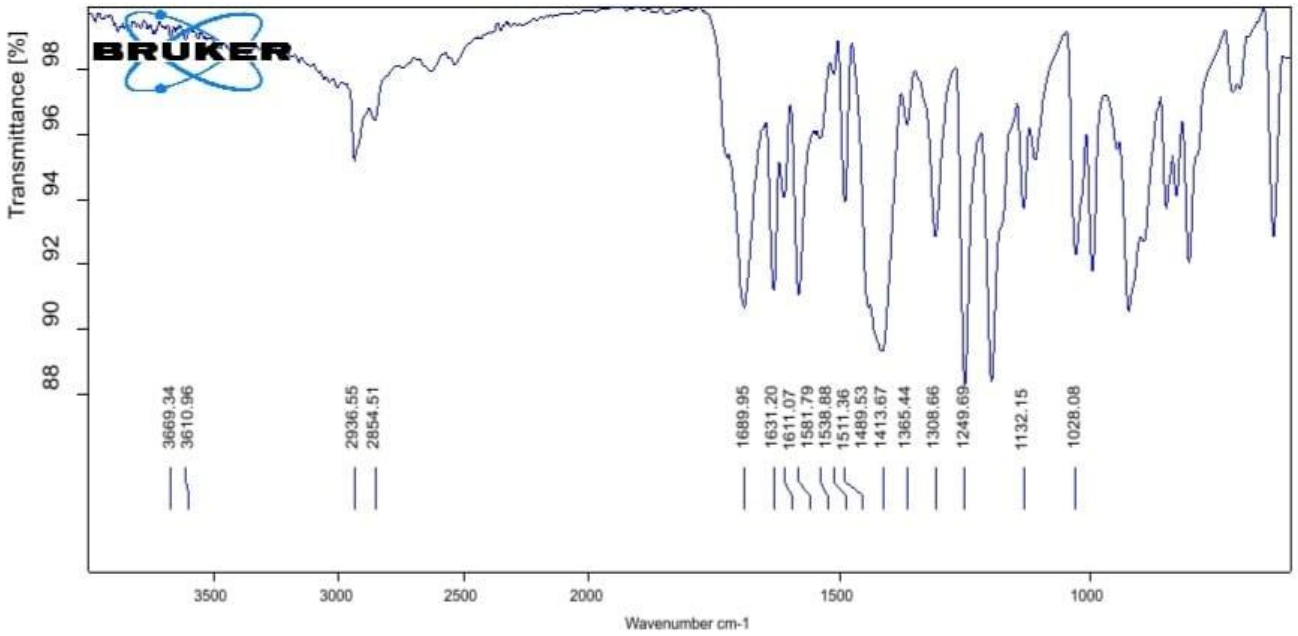

Fig 6: FT-IR of co-crystals.

Table no. 3: Functional groups observed in spectrum

S.No	**Piperine (**cm^{-1}**)**	**Succinic acid (**cm^{-1}**)**	**Physical mixture(**cm^{-1}**)**	**Co-crystal(**cm^{-1}**)**
1	Amide (C=O) 1574	C =O of (COOH) 1679	C =O piperine 1574	C =O 1581
2	Aromatic (C-H) 2926	O-H stretch 2663	C =O (succinic acid) 1679	C =O 1689
3	Aromatic (C =C)1493	C-H stretch 2949	C=C (piperine) 2931	C=C 2936
4	N-H 3406		C=C (piperine)1491	C=C 1489

Piperine exhibits characteristic FT-IR peaks at 1574 cm^{-1} (Amide C=O stretching), 2926 cm^{-1} (Aromatic C-H stretching), 1493 cm^{-1} (Aromatic C=C stretching), and 3406 cm^{-1} (N-H stretching). These peaks indicate the presence of functional groups such as carbonyl (C=O), aromatic carbon-hydrogen (C-H), aromatic carbon-carbon double bond (C=C), and amino (N-H) groups in the piperine molecule.

The co-crystal exhibits characteristic FTIR peaks C=O stretching vibrations at 1581 cm^{-1} and 1689 cm^{-1} indicative of two carbonyl groups; C=C stretching vibrations at 2936 cm^{-1} and 1489 cm^{-1}, representing two carbon-carbon double bonds; O-H stretching vibration at 2665 cm^{-1}, indicating the presence of hydroxyl groups; and N-H stretching vibration at 3610 cm^{-1} indicating the presence of amino groups. These peaks provide valuable insights into the molecular interactions and composition of the co-crystal.

In the FTIR analysis, co-crystals and piperine exhibited similarities, particularly in the presence of certain functional groups. Both substances showed peaks corresponding to the Aromatic (C-H) stretching vibration, indicating aromatic carbon-hydrogen bonds. Additionally, they displayed absorption peaks related to the C=C stretching vibration, suggesting the presence of carbon-carbon double bonds in their molecular structures.

4.2 Evaluation of Oral Thin Films

***Weight variation*:** Every film was weighed using an electronic balance. The weight of films was found to be 0.04, 0.06, 0.05, 0.05, 0.04, 0.05, 0.05, 0.05 (in gm). The average weight was found to be 0.048 gm. The weight variation was found to be minimum. It was demonstrated that the rise in the weight of the film is because of the increase in the concentration of the polymer.

***Flim thickness*:** The thickness of 10 films was found to be 0.14, 0.15, 0.13, 0.15, 0.13, 0.13, 0.10 mm. The average thickness result is 0.10 mm. The thickness of the films varies because of the quantity of the polymer within the formulation and since the polymer concentration within the initial films like Fl is incredibly less the films appear thin and as the concentration rises, the thickness increases as well. It was found to be within the vary of 0.10 mm – 0.14 mm. F3 formulation films were found to be clear and smooth.

FoldingEndurance: The endurance of the thin films was found within the range of 28-32. The films are flexible and strong enough.

***Appearance*:** Each film appeared clear, smooth, transparent and flexible.

***Drug content uniformity*:** The drug content uniformity is performed by taking 3 films in every formulation trial and therefore the average drug content was calculated and was found to be nearly 97%.

***In-vitro Disintegration time*:** The disintegration time of the prepared thin films was 40, 39, 35, 36 sec. The average result of disintegration time is 37.5 sec.

5. CONCLUSION

Piperine is a natural alkaloid found in black pepper (*Piper nigrum*, *Piper longum*) and it belongs to BCS class II. The preparation of a piperine co-crystal using succinic acid for use in oral films has potentially increased solubility and oral thin film is prepared with ideal properties.In this study, quite a few attempts were made within the formulation and evaluation of oral film containing piperine succinic acid co-crystal having polymer HPMC E15 and alternative excipients to formulate thin films of piperine and certain evaluations were carried out.The weight variation was found to be minimum. The thickness of the films was found to be uniform. Films were found to have sufficient strength. Disintegration time for the films was found within 37.5 sec. A Pediatric oral thin film was created by combining a piperine co-crystal with succinic acid as a conformer. To confirm the co-crystal formation, further evaluation is required to confirm co-crystal formation by differential scanning colorimetry and X-ray diffraction methods.

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