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DESIGN, DEVELOPMENT AND FORMULATION OF ECONAZOLE NITRATE CO-CRYSTAL LOADED GEL TO CURE TOPICAL ANTIFUNGAL DISEASE

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Keywords:

Co-crystal, Co-former, Econazole Nitrate, Synthon approach, Theoretical screening, Characterization

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ABSTRACT: In the past years many drug discoveries were investigated to be poorly soluble in aqueous medium. These poorly aqueous soluble drugs include Econazole Nitrate (ECZN) having high permeability. ECZN is an effective, potent anti-fungal drug used to cure large range of fungus. Oral administration of ECZN causes gastrointestinal irritation. To overcome or avoid the "first pass metabolism" and to optimise the drug concentration at the infected site of action topical gel was formulated, topical gels have better tendency to convey the drug *via* skin as compared to ointment, because gels are non-greasy and comfortable for application. To improve the solubility of ECZN in aqueous medium, Co-crystal technique was established to be a boon to the pharmaceutical industry. Crystalline structure of co-crystals consists of ECZN and Co-former either amino-acid or carboxylic-acid in equimolar ratio and penetration-enhancer was used. Liquid based grinding method used for fabrication of co-crystals. Formulated Co-crystal was incorporated in Carbomer-934 based topical gel. Characteristic peak was determined by Fourier transform infrared spectroscopy, the large number of particle size was found in the scale of 600- 1000 nm. Endothermic point was determined by thermal analysis (Differential scanning calorimetry) and Powder x-ray diffraction; microscopy of co-crystal was obtained by scanning electron microscopy. The formulated optimized co-crystal gel showed good potential of hydrogen, Viscosity, Zone of inhibition, drug content. ECZN co-crystal showed good in-vitro dissolution. Selected formulation of ECZN co-crystal loaded topical gel showed good ex-vivo permeability on topical skin and antifungal activity against *C. albicans*.

INTRODUCTION: Econazole Nitrate (ECZN) is a BCS class II weekly basic topical broad spectrum antifungal agent it has lower aqueous solubility that limits the penetration power and acts as barrier to topical drug delivery, ECZN approved by FDA in 26 November 2002. ECZN clinically used for the medication of topical fungal infections includes epidermal, dermal as well as deeper layers of skin and therefore it must be necessary to customize the drug delivery in all those layers.

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However, marketed formulations such as topical cream, ointment, powder affiliated with subordinate skin permeability and short retention time on skin of drug [1, 2]. ECZN shows its antifungal action by prohibiting the ergosterol synthesis, which is an important constituent of fungi cell membrane. It shows antifungal activity against fungi species such as *Candida albicans*, tinea-corporis, tinea-pedis [3].

Topical fungal infection and for skin care topical treatment of skin diseases a large range of commercially formulations are available but it requires more time for showing its result. So, the goal of current research study is to improve solubility of drugs in aqueous medium and incorporate them into the topical dosage form [4].

In the present era, to enhance the solubility of Biopharmaceutical classification system (BCS) class II drugs in the aqueous medium is the most challenging issue that has come into existence for pharmaceutical industries. More than or nearly 60-70% of recently discovered APIs belong to BCS class II and class IV having problems of solubility and permeability. Solubility of drugs can be enhanced by various methods such as physical modification (co-crystallization, size reduction, solid dispersion) and chemical modification (change in pH, complexation, *etc*.).

Over the past decades to reduce this problem there are numerous advanced drug delivery systems emerging in pharmaceutical industries such as formulating liposomes, noisome and applying nanotechnology, *etc*. However, although these methods could help to enhance the aqueous solubility of the drug, they may cause stability issues during storage or transportation due to atmospheric temperature, and may present other insurmountable difficulties. The first novel delivery of pharmaceutical Co-crystals invented by the German chemist Friedrich Wohler in 1844. He discovers Co-crystals quinhydrone by grinding of quinine with hydroquinone. A medicinal co-crystal is a multiphase crystal in which two ingredients are solid (API & co-former) and one component might be solvent which is additional component depend on the formulation technique and always have established stoichiometric ratio, such as 1:1 (1 mole of API:1 mole of co-former), 1:2 (1 mole of API:2 moles of co-former) and 2:1 (2 mole of API:1 mole of co-former) [5].

Which is invented by crystal engineering which were assembled from Hydrogen bonding, π-π stacking, Vander Waal's interaction, these all are the intermolecular interactions [6]. Co-former selection is the key role to synthesize the Co-crystals depending on the "synthon approach". It should have a proton donor and acceptor group which can be able to interact with hydrogen bonding as well as It should contain functional groups like carboxylic acid, amides, amines or alcohols which can produce strong hydrogen bonds [7]. If co-former has same therapeutic activity like API it is beneficial because in this case Co-crystals shows additive effect (In current research work ECZN is anti-fungal agent as well as Oxalic acid is also antifungal which gives better effect than only single Econazole nitrate). Pre-formulation studies conducted to determine physicochemical properties of drugs *e.g*. Solubility. Econazole nitrate compatibility with excipients have been checked at 25°C and 40°C. The different formulations of co-crystals were prepared by liquid based/ assisted grinding (LAG) technique with using different co-formers like Oxalic acid, L-proline, Succinic acid [8]. Co-crystals are superior to other methods in terms of chemical stability, toxicity, and ease of manufacturing.

In present research work, the prepared co-crystals of Econazole nitrate were incorporated into topical semisolid hydro gel for providing better drug permeability and retention time on skin at site of fungal infection. This research suggested the potential benefits of Econazole nitrate co-crystals incorporated in topical gel as drug delivery of drug by topical route for fungal infection treatment. This co-crystals system showed an overall 3-4 fold enhancement in solubility, higher penetration and optimum antifungal activity [9]. Gel incorporated with ECZN co-crystal could be a novel process with enhanced activity and improve delivery for APIs with poor aqueous solubility rather than crude drug containing gel. The objectives of current research work were to determine synthesis of co-crystals, solid state characterization, *In-vitro* release studies, *ex-vivo* permeability study, antifungal activity of ECZN co-crystals loaded topical gel formulation.

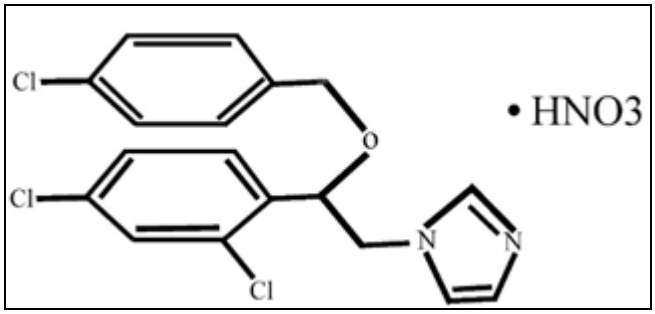

FIG. 1: STRUCTURE OF ECONAZOLE NITRATE

Synthon Approach / Hydrogen Bonding: Co-former selection is the key role to synthesize the co-crystals based on the "Synthon approach". Which constructs within the co-crystal a super molecule by utilizing specific molecular fragments to establish "supramolecular synthons. The basic necessity for a suitable selection of Conformer

which is pharmaceutically allowable, *i.e*. generally regarded as safe (GRAS) components. Current research work on hydrogen bonds forms between stable functional groups existing in the ECZN and co-former which plays a main role in the fabrication of crystal forms. Their reciprocal H^+ bond donor and acceptor spots makes supramolecular homo-synthon favourable. Hydrogen bonding interaction can be intramolecular or intermolecular interaction. Conformers should have a proton acceptor and donor group which can interact with hydrogen bonding with ECZN. It should contain functional groups like amines, amides, carboxylic acid, and alcohols which can produce strong hydrogen bonds. Common hydrogen bond produced from carboxylic acids, pyridines, amides and other aromatic nitrogen. Hydrogen bonding or synthon approach can be determined by the FTIR spectrum of ECZN co-crystals due to unavailability of Cambridge expert software [10–12].

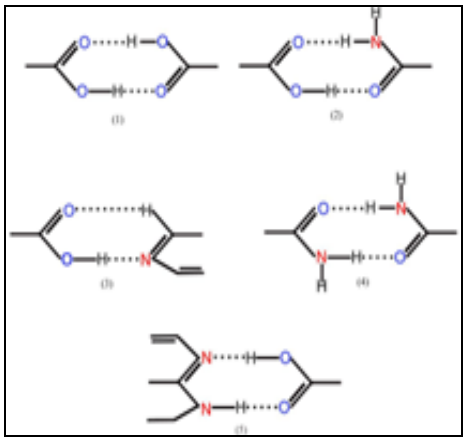

FIG. 2: SYNTHON APPROACH (HYDROGEN BONDING) BETWEEN TWO SOLID COMPONENTS

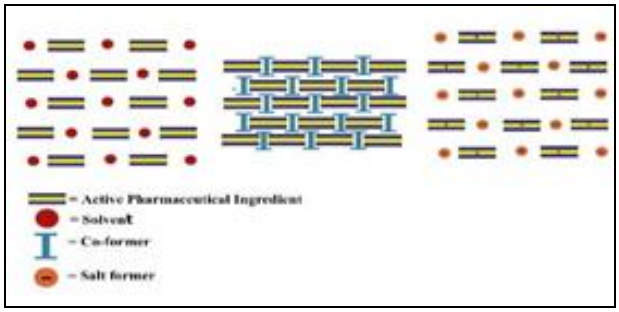

FIG. 3: SCHEMATIC REPRESENTATION OF APIS, SOLVATES/HYDRATES, CO-CRYSTALS AND SALTS

Theoretical Screening for Co-Crystallization:

pKa Approach: Simple and prominent approach to identification of co-crystal formation. A salt is obtained if the difference between the pKa base and pKa acid (δ pKa) is >3, whereas a δ pKa <0 will generally result in the fabrication of a co-crystal. ΔpKa (δ pKa = pKabase-pKaacid) must be less than 0 [5].

Prediction of Co-crystal Fabrication by Theoretical Hoy's Molar Attraction Method: For calculation constant functional groups values found from the various literatures [13].

Formula to calculate Hoy's molar solubility parameters (δ) = Molar attraction constant / Molar volume constant [5].

Preformulation Studies:

Solubility Determination: The solubility of ECZN in various solvents was analysed. For this an excess amount of Econazole nitrate was added to volumetric flasks containing 5ml of the different solvents in 5ml capacity vials separately and the mixture was mixed by vortex mixer till saturation. The vials were shaken on a mechanical shaker for 48 hours followed by centrifugation keeping speed 3,000 rpm for 30 min. The solution of each sample was filtered through cellulosic Whatman filter paper. Obtained Filtrate was diluted same respective solvents as required and analysed using UV spectrophotometer. The components that exhibited the highest solubility of ECZN were used for further studies.

Melting Point Determination of Each Ingredient: Melting point is the temperature at which the pure liquid and solid exist in equilibrium. In practice, it is taken as an equilibrium mixture at an external pressure of the atmosphere; this is sometimes known as normal melting points. The sealed capillary method is used, by introducing a minute amount of drug into a small thin-walled capillary glass tube sealed at one end. A packed capillary was attached to a normal mercury thermometer then capillary was inserted into a digital melting point equipment and the temperature was observed at which melting starts and is complete.

Method Development and Validation:

Determination of Wavelength of Maximum Absorption: The UV spectrometric method was developed and validated as per ICH Q2 (R1) guidelines [14]. The change in λ_{max} of the drug ECZN was observed with a change in the solvent system. Therefore, it was necessary to analyse drugs at

different wavelengths in different solvents. Wavelength selection for method A and method B proceeded as follows:

For Method A: A standard stock solution of ECZN (100 µg/mL) was prepared using methanol, 1 mL of stock solution was then diluted up to 10 mL with the same solvent to obtain 10 µg/mL ECZN solution. Prepared dilutions were scanned between 200 nm to 400 nm, using the methanol as the diluents for the blank. The wavelength of maximum absorbance of ECZN was found to be 220nm.

For Method B: Quantification of ECZN in Phosphate buffer, the standard stock solution of Econazole Nitrate (100 µg/mL) was prepared using PBS pH 7.4: methanol. Prepared solutions were scanned between 200 nm to 400 nm, using PBS pH 7.4 + methanol as a blank. The wavelength of maximum absorbance of ECZ was found to be λmax= 265nm.

Method of Preparation of Co-Crystals:

Neat Grinding Method: API and different individual co-formers were accurately weighed and carried out the physical samples to laboratory mortar pestle for grinding. Samples were taken out at the intervals of 15 min- 45 min of grinding and analysed for Melting point, FTIR, DSC, PXRD and SEM.

Solvent Evaporation Method: API, Different individual Co-formers dissolved in solvent (DMSO). After making a solution, evaporate the solvent using a magnetic stirrer or hot plate. After evaporation co-crystals were collected.

Liquid Assisted Co-grinding: API and different co-formers were accurately weighed and transferred to mortar pestle with addition of solvent (DMSO), DMSO significantly increases penetration in topical pharmaceutical preparations. Samples were carried out at the intervals of 15 min-45 min of grinding and characterized for Melting point, FTIR, DSC, PXRD and SEM.

Selected Method of Co-crystals: Among these three methods, the Liquid assisted co-grinding gave better results as compared to the Neat grinding method and Solvent evaporation method.

Liquid assisted co-grinding is a green method. According to literature formulated, co-crystal's melting point was found to be less than the melting point of API and melting point of co-former and it gives better penetration after incorporation in topical hydrogel. Solvent evaporation method not suitable for small batch production.

Method of Preparation of Topical Gel: Propyl-paraben and methyl-paraben were taken as a preservative in a beaker containing propylene glycol. Above ingredients were mixed properly by gentle stirring (100 rpm) and raising the temperature till 50°C on a hot magnetic stirrer. HPMC, Carbopol- 934, carbomer-934 was dispersed slowly into separate beaker containing a sufficient quantity of distilled water and allowed to soak for 4-5 hours. Soaked HPMC, Carbopol- 934 and carbomer-934 was stirred separately for about 30min at 600 rpm followed by the addition of glycerine and polyethylene glycol 400, Econazole nitrate, tween 80 and ethanol were taken in a centrifuge tube which was vortexed for 15min the centrifuged solution was mixed with previously prepared polymeric solution by vigorous stirring at 1200rpm for 15min with the help of a mechanical stirrer. Triethanolamine use as a pH adjuster which was added drop wise till obtain the final mixture and stirred thoroughly until viscous homogeneous gel was obtained [15, 16].

TABLE 1: FORMULA OF TOPICAL GEL

Component	Quantity taken							
	GB1	GB2	GB3	GB4	GB5	GB6	GB7	GB8
Econazole Nitrate	1%	1%	1%	1%	1%	1%	1%	1%
Carbomer- 934	0.05% w/v	0.75% w/v	1% w/v	1.5% w/v	-	-	-	-
HPMC	-	-	-	-	0.05% w/v	0.75% w/v	1% w/v	1.5% w/v
Propylparaben	0.5% w/v	0.5% w/v	0.5% w/v	0.5% w/v	0.5% w/v	0.5% w/v	0.5% w/v	0.5% w/v
Methylparaben	0.1% w/v	0.1% w/v	0.1% w/v	0.1% w/v	0.1% w/v	0.1% w/v	0.1% w/v	0.1% w/v
Propylene glycol	1.0% v/v	1.0% v/v	1.0% v/v	1.0% v/v	1.0% v/v	1.0% v/v	1.0% v/v	1.0% v/v
Glycerine	3% v/v	5% v/v	10%v/v	10%v/v	3% v/v	5% v/v	10%v/v	10%v/v
Polyethylene	5% v/v	5% v/v	5% v/v	5% v/v	5% v/v	5% v/v	5% v/v	5% v/v

glycol- 400								
Tween 20	2% v/v	2% v/v	2% v/v	2% v/v	2% v/v	2% v/v	2% v/v	2% v/v
Tween 80	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v	1% v/v
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Distilled water	100ml	100ml	100ml	100ml	100ml	100ml	100ml	100ml

Evaluation of Co-Crystal Loaded Topical Gel:

Evaluation of Co-crystals:

Fourier Transforms Infrared Absorption Spectrum of Pure API: Fourier Transmission Infra-Red Spectroscopy (FTIR) of the drug was performed using Shimadzu Bhanuben Nanavati College of Pharmacy, Mumbai. IR Affinity in the range of wave number 500 to 3500 cm^{-1}. The FTIR spectrum of the drug was taken by using the ATR method. It was compared with the reference spectrum and the peaks were found according to presented functional groups in the drug [17, 18].

Zeta Potential: The zeta potential value of co-crystal was obtained by Malvern Zetasizer ZS at Bhanuben Nanavati College of Pharmacy, Mumbai. ECZN co-crystal was diluted 100 times with double distilled water and voltage was set at 1.4 V and electrodes were placed in dispersion for measurement of zeta potential. Each sample was run 3 times and analysis was continued out at 25°C with scattering angle or 173°C.

***In-vitro* Dissolution Study:** *In-vitro* powdered drug release study of Co-crystal was carried out by using a mixture of acetate buffer pH 5.5: methanol (7:3), the dissolution vessel was filled with 500ml of mixture. The Co-crystal equivalent to 10 mg was packed into the muslin cloth. Packed muslin cloth was put inside the basket and attached in a vessel containing a mixture of acetate buffer solution pH 5.5: Methanol which acted as receptor compartments.

The RPM was set at 100/min and temperature was maintained at 37°C. At predetermined intervals of 5 hrs samples were withdrawn and replaced with 5ml of the same mixture solution to maintain sink condition throughout the experiment. Samples withdrawn were diluted and analysed by UV spectrophotometer [19, 20].

Differential Scanning Calorimetry (DSC) of Pure API: Differential scanning calorimetry experiments were performed on a differential scanning calorimeter SIECKO (NMIMS), SII nanotechnology DSC-500 in the dynamic mode at a healing rate of 10°C/min (gas argon flow rate at 50mL/min) and with the temperature range 50-330°C to determine the exothermic or endothermic pattern of complexes [21–25].

Scanning Electron Microscopy (SEM): The surface morphology of ECZN Co-crystals was studied by SEM. Shape of formulation was determined by SEM (Diya Labs). The sizes of the Co-crystals were measured by scanning electron microscopy. Small amount of Co-crystals was placed on the specimen stub, coated with carbon and then with gold vapour using Hitachi vacuum evaporator. The samples were examined under scanning electron microscope for vesicular shape, and then photographed.

Evaluation of Co-crystals:

Physical Appearance and Uniformity: The physical appearance and homogeneity of the formulated ECZN co-crystal loaded gels was tested by visually after the gels have been settled in the container. They were examined for their appearance and uniformity.

Viscosity: The evaluation of viscosity of the formulated co-crystal loaded gel was done with Brookfield Viscometer (DV-E). 10 gm of gel was taken in a glass container and the spindle number 64 was immersed into the gel formulation at 12 rpm because gel comes under the HA (high viscosity) category.

Measurement of pH: The pH was measured by calibrated digital pH meter, readings were taken in triplicate by dipping the glass electrode into the gel formulations.

Spreadability: The Spreadability was detected using the following method: 1g of topical gel was placed between a circle on a glass slide block on which an another glass slide was placed. A load of 50 g was placed to rest on the upper glass slide block. The shifting of above glass slide was observed.

The scale was measured in cm and time was determined in seconds this method was performed to check the Spreadability. The mean diameter was taken by repeating the experiment three times 77, 85.

$$S = m \times L/t$$

Where, S: Spreadability, M: weight of load, L: length travel by upper slide, T: time.

Extrudability: The extrusion measurement of the ECZN topical gel from the aluminum collapsible tube is an important characteristic during its application on skin and patient compliance. This analysis is useful to check whether the ECZN co-crystal loaded gel is easily coming out from the aluminum collapsible tube during application in appropriate manner or not. Highly viscous gel may not be coming out from the tube whereas, low viscous gels coming out quickly, and hence suitable viscosity is required in order to extrude the gel from the collapsible tube. The gel was filled into aluminum collapsible tubes. The tubes were pressed to extrude the ≈0.5 cm band of the gel in 10 seconds and the Extrudability of formulations was measured.

***In-vitro* Permeability Study:** *In-vitro* permeability study of Co-crystal loaded GB2, GB3, GB4 and GB8 topical gel batches was carried out by using a mixture of phosphate buffer pH 7.4: methanol (7:3) on Franz diffusion cell. The dialysis membrane was used as an artificial membrane instead of animal skin. The receptor compartment was filled with 20ml of prepared solvent mixture. The RPM was set at 100/min and temperature was maintained at 37°C. At predetermined intervals of 6 hrs samples were withdrawn and replaced with equivalent amount of the same mixture solution to maintain sink condition throughout the experiment. Samples withdrawn were diluted and analysed by UV spectrophotometer [20, 26].

***Ex-vivo* Permeability Study on Goat Skin:** Skin permeability study was performed using a Franz permeability determined cell with a diffusion area of 2.5 cm^2. The practical was carried out using fresh goat abdominal region of skin and stored at –25°C in the physiological salt solution. Skin was first flushed with a physiological solution at 25°C for 2hours to remove any skin content. The skin hairs were removed using a scissor and hand razor to remove. To remove excess fat on subcutaneous tissue and the dermis side was cleaned with isopropyl alcohol. The cleaned skin layer was cleaned or washed with distilled water. A circular piece of skin (about 2.5 cm diameters) was sandwiched between donor and receptor compartment of the vertical diffusion chamber and ECZN Co-crystal loaded 1gm topical gel was placed to the topical side of skin. In the donor compartment, the formulation was placed in intimate contact with the skin. The phosphate buffer pH 7.4: methanol (7: 3) filled into the receptor compartment, kept at constant temperature of 37 ± 05ºC and stirred using a magnetic stirrer. At specific time intervals (1, 2, 3, 4, 5, 6, 16 to 24 hr), 2 ml aliquots of the receptor medium were withdrawn and replaced by a same quantity of fresh receptor solution. Samples were analysed by UV Spectrophotometer (Shimadzu-1800, Japan) at 265 nm using phosphate buffer (pH 7.4): methanol (7: 3) as blank. Permeation studies of marketed gel were also investigated in the same way [27, 28].

Antifungal Studies: *Candida albicans* slant (ATCC 10231) was obtained from the microbiology department of the Naprodlifesciences, Mumbai. 5ml of Sabouraud Dextrose Broth (SDB) was added to the slant and incubated for 24 hrs for growth of Candida albicans. 2.6gm of Sabouraud dextrose agar (SDA) was added in 40ml distilled water in conical flask and stoppered with cotton plug and autoclaved for 15 min at 15 lbs pressure. Then cooled at room temperature. Pour 20ml into three sterile Petri plates in laminar air flow, swirl the plates to remove air bubbles and let it solidify at room temperature near the sterile area. Add of *C. albicans* fungi and spread in all directions using spreader into the solidified agar, bore three wells from the sterile borer to add co-crystal, excipient, pure drug, ECZN co-crystal loaded topical gel, blank solvent & marketed formulation. Incubate at 25-30°C for 48 hr for inhibition of fungi and observe MIC (*i.e.* - zone of inhibition) and measure diameter in mm; more the ZOI the more susceptible the formulation is against MF [29].

Stability Study: Stability of co-crystal suspension was performed as per ICH Q1 (R2) guidelines for 3 months [30]. Sufficient quantity of the co-crystal gel

formulations was sealed in a 10g collapsible aluminium tube. The samples were withdrawn at each month entire duration of 3 months and leakage of drug from the formulation was analysed for drug content by using a UV spectrophotometer.

RESULTS AND DISCUSSION:

Melting Point:

TABLE 2: DETERMINATION OF MELTING POINT

Sr. no.	Name of Ingredients	Observed Melting Point of individual component	Observed Melting Point of formulated co-crystal (API: Co-former in the ratio of 1:1)	Observed Melting Point of formulated co-crystal (API: Co-former in the ratio of 1:2)
1	API (Econazole Nitrate)	164°C	-	-
2	Succinic acid	180°C	115°C	95°C
3	Serine	222°C	145°C	110°C
4	Oxalic acid	98°C	88°C	75°C
5	L-Proline	204°C	165°C	125°C
6	Aspartic acid	275°C	210°C	160°C
7	Ascorbic acid	191°C	140°C	95°C
8	Glycine	235°C	180°C	135°C

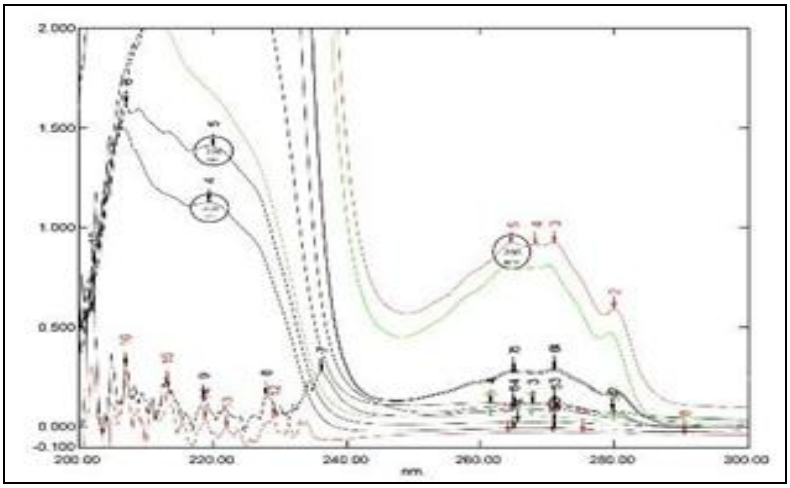

FIG. 4: SPECTRUM OF ECZN IN DIFFERENT SOLVENTS

From the table melting point of all the formulated co-crystal was observed less then melting point of API and co-former. This result confirms that the formulated co-crystals are stable.

Method Development and Validation: The developed UV method for the quantification of Econazole Nitrate was validated as per the ICH Q2(R1) guideline. Different parameters like linearity, accuracy, precision, robustness, the limit of detection (LOD), and limit of quantitation (LOQ) were evaluated.

Solubility: Econazole nitrate was freely soluble in methanol, dimethyl sulfoxide, partially soluble in ethanol, slightly soluble in PBS (pH 7.4) and insoluble in Water. + + + + freely soluble 1-10 parts + + + soluble 10-30 parts + + slightly soluble 100-1000 parts + Insoluble 10,000 and above.

TABLE 3: LINEARITY OF ECONAZOLE NITRATE

Sr. no.	Concentration µg/mL	Mean
1	2	0.1243
2	4	0.3603
3	6	0.5723
4	8	0.8266
5	10	1.0233

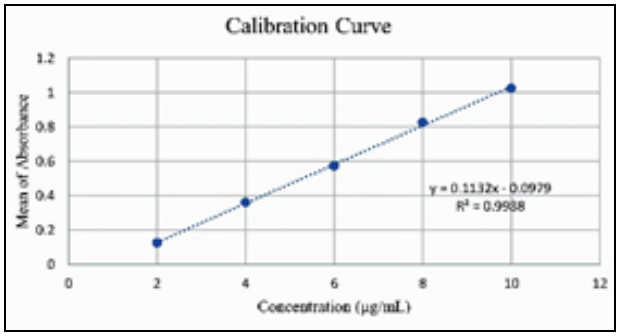

FIG. 5: PLOT OF LINEARITY (METHOD A)

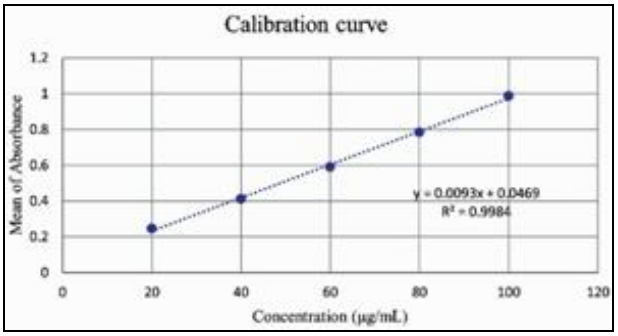

FIG. 6: PLOT OF LINEARITY (METHOD B)

TABLE 4: LINEARITY OF ECONAZOLE NITRATE

Sr. no.	Concentration µg/mL	Absorbance
1	20	0.245
2	40	0.411
3	60	0.59
4	80	0.784
5	100	0.9856

TABLE 5: RESULT OF METHOD VALIDATION USING UV SPECTROMETRY

Validation parameters	Acceptance criteria	Solvent system	
		Methanol	Phosphate buffer pH 7.4+30% Methanol
Linearity	Regression coefficient should be NMT or near to 1	0.9988	0.9984
Range	NA	1-10 µg/ml	10-100µg/ml
Slope	-	0.1132	0.0093
Intercept	-	0.0979	0.0469
Limit of detection (LOD)		0.9681	11.23
Limit of quantification (LOQ)		2.9338	34.03
Accuracy Mean % recovery (n=3), 80%, 100%, 120%	Between 80-120%	80%, 87%, 89%	89%, 88%, 85%
Precision – Repeatability, Average %RSD	%RSD NMT 2.0%	0.33%	0.53%
Intraday precision, Mean absorbance %RSD		0.12%	1.32%
Interday precision, Mean absorbance %RSD		0.47%	0.81%
Robustness, pH 7.4, Wavelength 265nm		- - 219nm=0.77% 221nm=0.81%	pH7.3= 0.45% pH7.5= 0.73% 264nm= 0.35% 265nm= 0.11%

FTIR: The FTIR shows important peaks due to functional groups of ECZN and co-formers are present in Co-crystals.

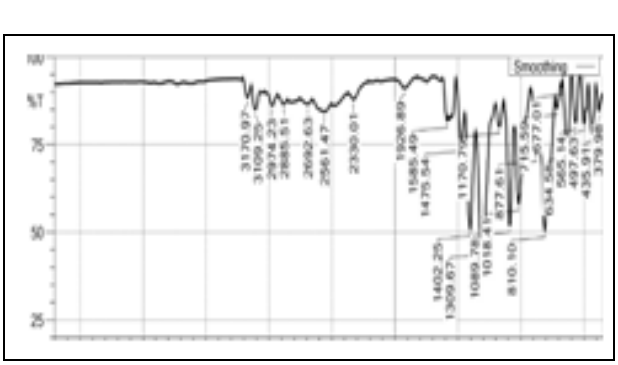

FIG. 7A: FTIR SPECTRUM OF ECZN

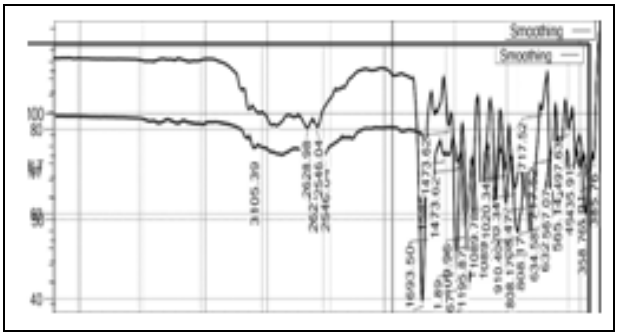

FIG. 7B1: FTIR SPECTRUM OF ECZ: OXALIC ACID (1: 1)

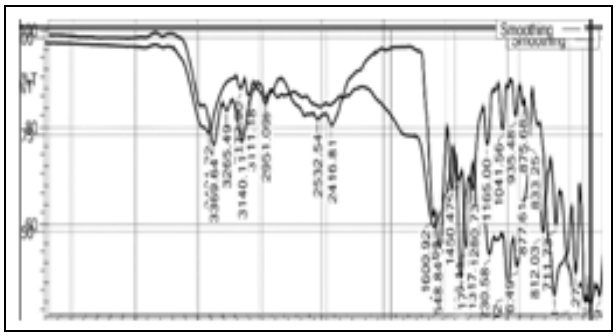

FIG. 7B2: FTIR SPECTRUM OF ECZ: OXALIC ACID (1: 2)

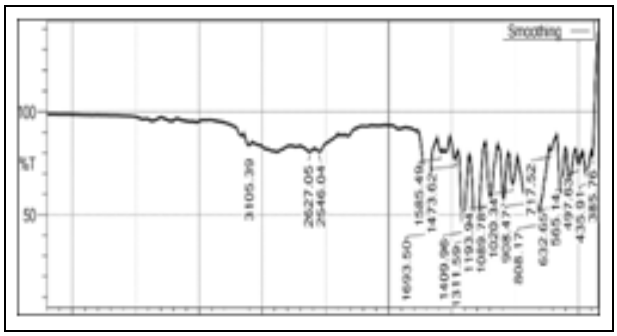

FIG. 7 (C1): FTIR SPECTRUM OF ECZ: SUCCINIC ACID (1: 1)

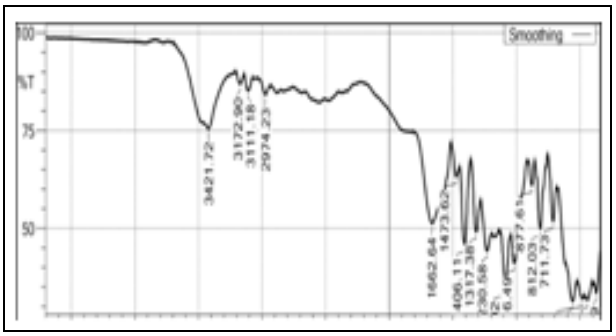

FIG. 7 (C2): FTIR SPECTRUM OF ECZ: SUCCINIC ACID (1: 2)

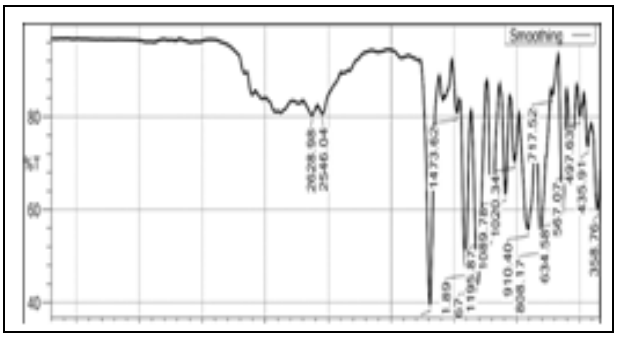

FIG. 7 (D1): FTIR SPECTRUM OF ECZ: L-PROLINE (1: 1)

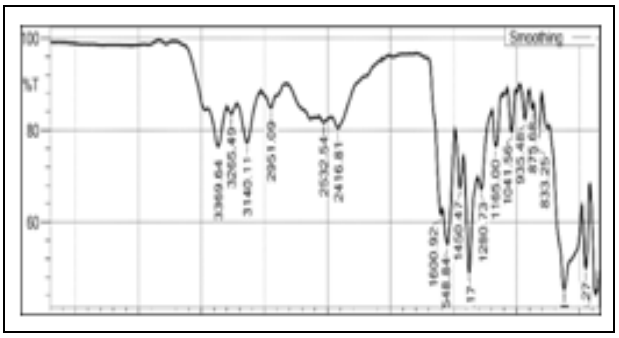

FIG. 7 (D2): FTIR SPECTRUM OF ECZ: L-PROLINE (1: 2)

The FTIR spectrum of ECZN with Succinic acid, L-proline and oxalic acid (1: 1) co-crystal does not show any new peak that determined the hydrogen bond was not formed between API and co-former. ECZN with Succinic acid, L-proline and oxalic acid (1: 2) co-crystal showed a new peak that determined the hydrogen bond was formed between API and co-formers.

Peaks was observed in the range of 3000-2850 cm-1indicate the presence of C-H stretching, 1600-1475 cm^{-1} indicate the presence of C=C stretching, 1300-1000 cm^{-1} indicate the presence C-O stretching, 1350-1000 cm^{-1} indicate the presence of C-N stretching, 700-900 cm^{-1} indicate the presence of C-Cl, 1550 & 1350 cm^{-1} indicate the presence of NO_2 group 2500-3500 cm^{-1} indicate the presence of OH stretch suggesting the presence of hydrogen bonding.

These observed peaks show distinct characteristics of carboxylic acid with NH or OH groups. 400-800 cm^{-1} indicate the presence of halogen- hydrogen interacting bonds that denote that the synthon formed between Cl and OH.

Zeta Potential: Zeta potential is an index which indicating particle size distribution of relative co-crystal particle amount as a percentage where the total amount of particle is 100%.

Different particle size ranges for different route of administration is as follows:

TABLE 6: SUITABLE RANGE OF PARTICLE SIZE FOR DIFFERENT ROUTES

Route of administration	Particle size distribution range
Stratum corneum	>600 nm
Dermal	>10 nm or <600 nm
Ophthalmic	>100 nm or <3000 nm
Nasal	>8 µm or <12 µm
IV/ IM	>200 nm or <2000 nm
Aerosol	>1 µm or < 10 µm
Brain or Tumour	>50 nm or < 200 nm

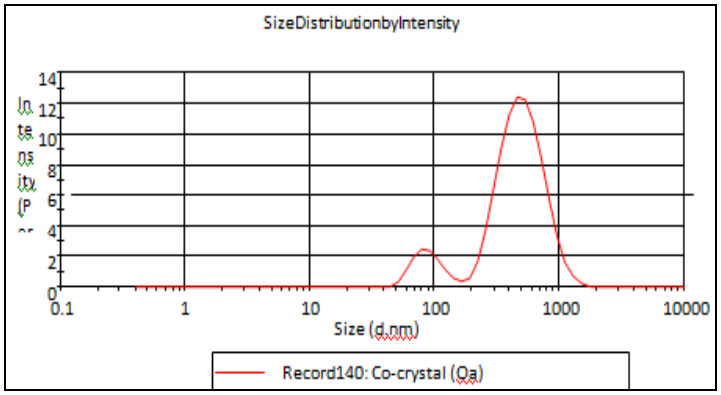

FIG. 8: SPECTRUM OF ZETA POTENTIAL

The 88% size distribution of optimized co-crystal formulation was found in the range of 628.3 nm and 12% was found in the range of 90.46%.

PDI was found to be 0.362, which is under the standard limit <0.5, revealing homogeneous distribution.

***In-vitro* Powder Dissolution Study:** The dissolution study of ECZN: Oxalic acid co-crystal, ECZN: L-proline co-crystal, ECZN: succinic acid co-crystal and pure ECZN was performed triplicate on USP type I basket apparatus to determine the release of drug outside the body.

TABLE 7: *IN-VITRO* DISSOLUTION STUDY

Time (min)	%CDR (Oxalic acid CC)	%CDR (L-Proline CC)	%CDR (Succinic acid CC)	% CDR (Free drug)
0	0	0	0	0
15	12.67442	5.697674	8.023256	5.697674
30	17.57907	8.137209	10.5093	8.137209
45	22.32326	12.83488	12.8814	10.5093
60	25.90465	19.90465	17.57907	12.8814
120	31.78837	25.85814	23.48605	19.90465
180	34.23023	29.46279	31.74186	24.69535
240	40.0907	35.34651	38.8814	29.43953
300	57.64884	52.90465	55.3	34.18372

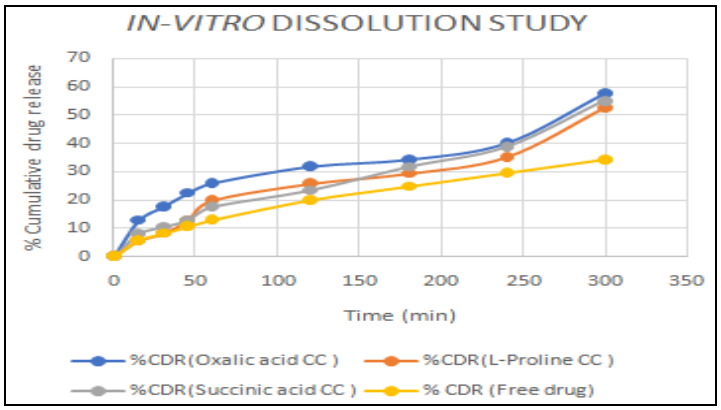

FIG. 9: PLOT OF *IN-VITRO* DISSOLUTION STUDY

The dissolution rate of co-crystals increases as compared to pure ECZN. ECZN: Oxalic acid co-crystal > ECZN: L-proline co-crystal > ECZN: Succinic acid co-crystal > Pure ECZN. Formulated co-crystal of ECZN with oxalic acid gives better dissolution rate.

DSC: The study DSC was conducted to determine molecular dispersion of ECZN into optimized co-former Oxalic acid. DSC thermogram of ECZN, Oxalic acid and formulated co-crystal were compared.

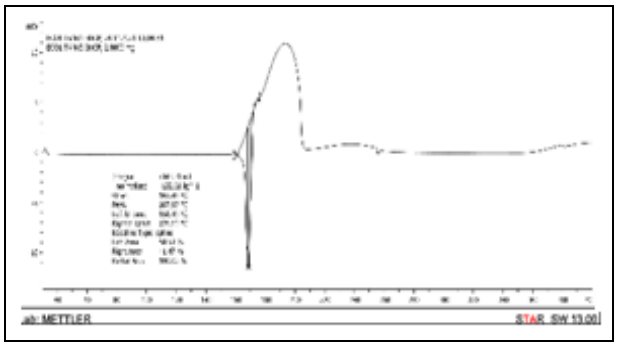

FIG. 10A: DSC SPECTRUM OF ECZN

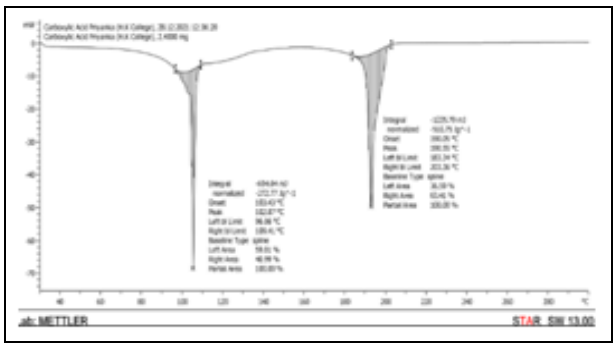

FIG. 10B: DSC SPECTRUM OF OXALIC ACID

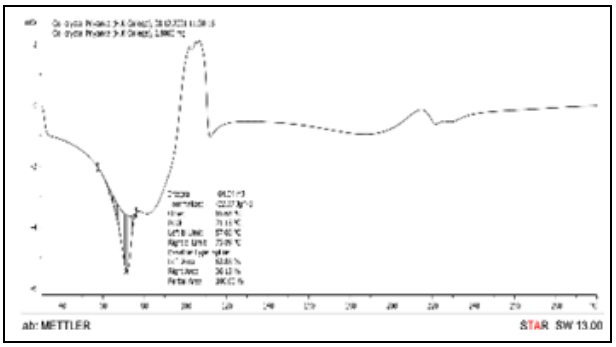

FIG. 10C: DSC SPECTRUM OF ECZN CO-CRYSTAL

The pure ECZN showed endothermic peak at 167.97°C. The pure oxalic acid showed endothermic peak at 102.87°C and the formulated co-crystal showed endothermic peak at 71.15°C which is less then endothermic peak of pure ECZN and co-former.

PXRD: The PXRD pattern of the ECZN and Co-crystal showed in following figure the PXRD spectrum of formulated co-crystals showed intense endothermic peaks as compared to PXRD of pure ECZN. Co-crystal showed crystallinity at 13.927°, 14.9484°, 16.4471°, 18.2795°, 19.2651°, 20.423°, 22.8121°, 23.3331°, 24.592°, 26.5421°, 28.249°, 29.0095° (2θ) with peak intensities of 44.64, 474.34, 13.75, 28.6, 29.2, 144.9, 107.59, 142.73, 90.08, 162.13, 38.32 and 271.86 in height (counts) indicating its crystalline nature.

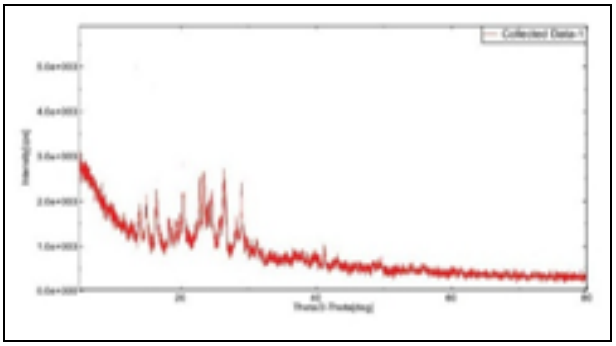

FIG. 11 (A): PXRDSPECTRUM OF ECZN

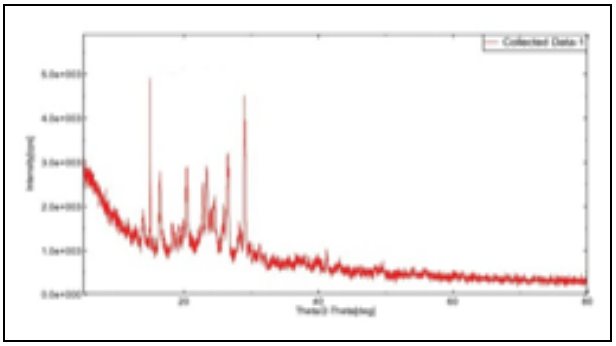

FIG. 11 (B): PXRDSPECTRUM OF ECZN CO-CRYSTAL

SEM: The SEM study was conducted at nano-scale to identify the shape and structure of co-crystals.

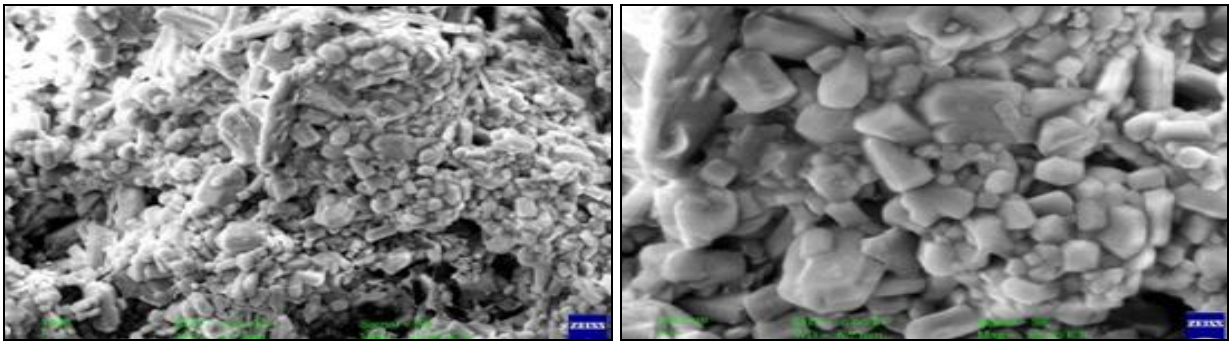

FIG. 12: STRUCTURE OF CO-CRYSTAL

The shape and structure of ECZN co-crystal exhibited smooth, homogeneous, irregular in shape.

Measurement of Viscosity, Spreadability and pH

Formulations	Viscosity(cps)	Average Spreadability
GB1	27740	30.95
GB2	38110	28.75
GB3	38190	29.04
GB4	49140	20.83
GB5	18900	46.66
GB6	18290	47.91
GB7	29910	35.71
GB8	29990	35

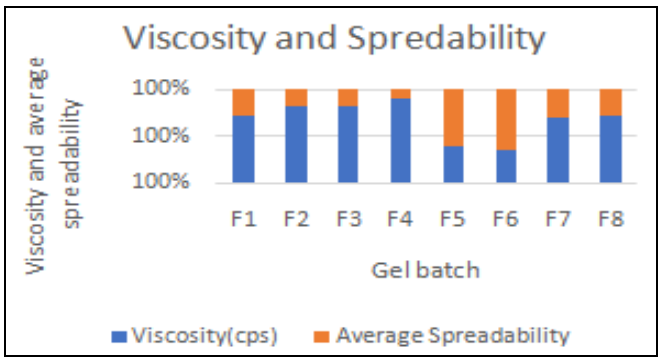

FIG. 13: MEASUREMENT OF VISCOSITY AND SPREADABILITY

From the result the GB2, GB3, GB4, GB8 represents better viscosity and good Spreadability as compared to other batches. pH 5.5 kept for topical gel.

Extrudability:

FIG. 14: ESTIMATION OF EXTRUDABILITY

TABLE 9: RESULT OF EXTRUDABILITY

Sr. no.	Formulation Code	Extrudability (cm)
1.	GB2	1.3cm
2.	GB3	1cm
3.	GB4	0.6cm
4.	GB8	2cm

From the above result we can conclude that in 14 seconds the Extrudability of Gel Batch 2, 3 and 4 was found to be near to the Extrudability of marketed formulation.

***In-vitro* Permeability Study:** *In-vitro* permeability study of gel batches was performed triplicate for 6 hours using dialysis membrane on Franz diffusion cell.

TABLE 10: *IN-VITRO* DRUG PERMEABILITY STUDY

Time	B2 %CDR	B3 %CDR	B4 %CDR	B8 %CDR	Standard %CDR
0 min	0	0	0	0	0
15min	15.9354839	15.5053763	10.7741935	3.03225806	3.89247312
30min	21.4	17.916129	15.2924731	6.1311828	5.57204301
60min	23.2924731	19.7225806	17.3569892	9.63655914	8.28172043
120min	24.5182796	23.7655914	20.5397849	11.6795699	9.61505376
180min	28.0666667	26.9483871	22.1311828	13.572043	10.3677419
240min	31.6365591	29.1634409	26.1311828	15.4645161	14.3032258
300min	35.1849462	32.1526882	26.9483871	18.6473118	16.1956989
360min	37.227957	35.227957	28.711828	21.9591398	18.2817204

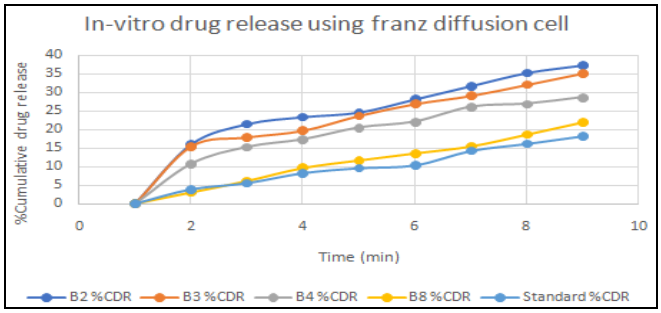

FIG. 15: PLOT OF *IN-VITRO* PERMEABILITY STUDY

The topical gel batch 3 gives better *in-vitro* drug permeability as compared to gel batch 2, 4 and gel batch 8. The study was conducted for 6 hour's artificial dialysis membrane.

***Ex-vivo* Study:** The *ex-vivo* study was conducted on goat skin to determine permeability of co-crystal loaded topical gel in PBS pH 7.4.

FIG. 16: *EX-VIVO* STUDY

TABLE 11: *EX-VIVO* PERMEABILITY STUDY USING ANIMAL SKIN

Time	%CDR of GB3	%CDR of MF
0 min	0	0
15min	1.956989	0.451613
30min	3.443011	1.356989
60min	4.217204	2.088172
120min	5.141935	3.227957
180min	6.948387	4.625806
240min	9.055914	5.830108
300min	11.18495	6.582796
360min	14.38925	8.152688

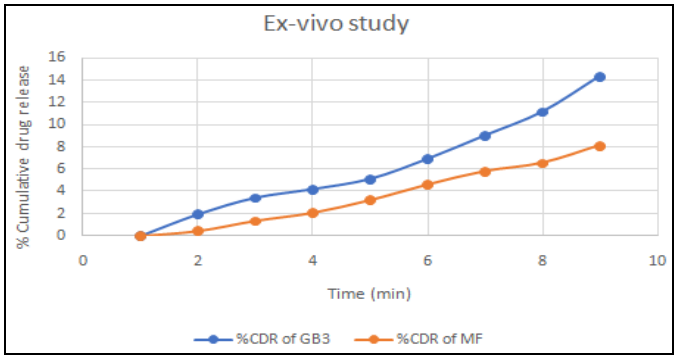

FIG. 17: PLOT OF *EX-VIVO* PERMEABILITY STUDY

The analysed absorbance was determine the drug was permeable from the topical gel batch 3 and transverse from the goat skin into the PBS pH 7.4 which was artificial solution used instead of blood. From the calculation %CDR of ECZN gel batch 3 was found to be 7.44% at 1440 min and %CDR of marketed formulation was found to be 6.79% at 1440min in PBS pH 7.4.

Antifungal Study: The antifungal activity of pure Econazole Nitrate, Econazole Nitrate co-crystal, ECZN co-crystal loaded topical gel, co-former, blank solvent, marketed formulation was studied **Table 11.**

FIG. 18: ZONE OF INHIBITION

TABLE 12: ZONE OF INHIBITION

Zone of inhibition (mm) ± SD	Formulation					
	Pure ECZN	Co-crystal	Blank	ECZN co-crystal loaded topical gel	Oxalic acid	Marketed formulation
	8 ± 0.1527	12 ± 0.1732	4.3 ± 0.1527	10 ± 0.1527	5 ± 0.1154	7.6 ± 0.0577

Stability Study: According to above results, stability study was conducted only for final optimized gel batch at 25°C ± 2°C and 40°C ± 2°C, short term and accelerated studies respectively, for the period of 3 months. The ECZN co-crystal loaded topical gel was analysed for drug content and *in-vitro* drug release. There was a minor decrease in all the parameters.

TABLE 13: STABILITY STUDY OF GB 3 AT 25°C

Time (Months)	Drug content (mg)	% Remained	Log % Remained	%DC
0	10	100	2	100
1 month	9.64	90.36	1.9840	96.4
2 month	9.52	90.48	1.9786	95.2
3 month	9.50	90.50	1.9777	95.0

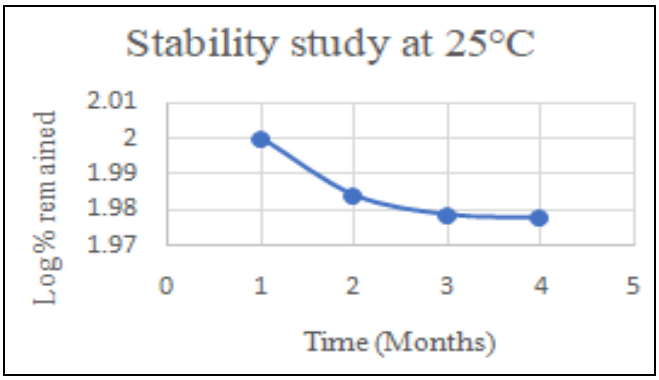

FIG. 19(A): PLOT OF STABILITY STUDY AT 25°C

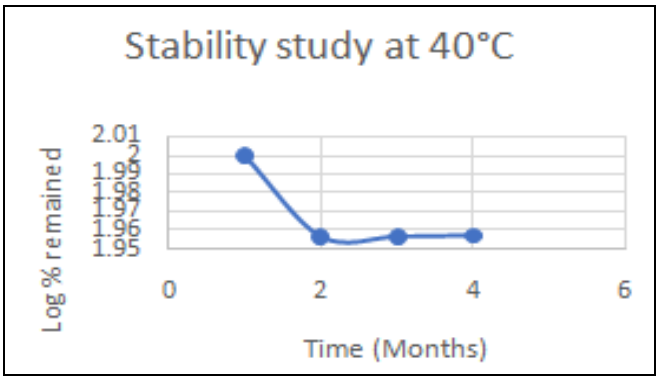

FIG. 19(B): PLOT OF STABILITY STUDY AT 40°C

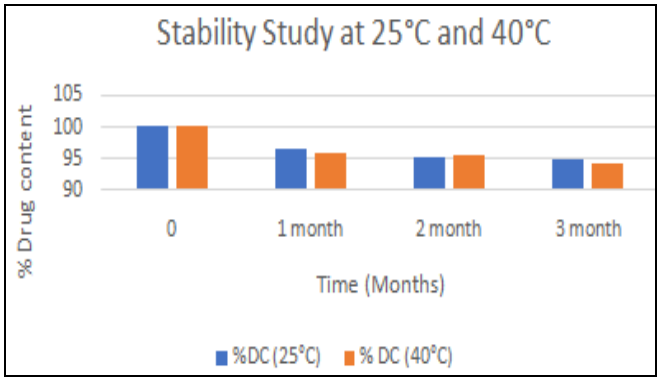

FIG. 19(C): %DRUG CONTENT AT 25°C AND 40°C AFTER THREE MONTHS OF CO-CRYSTAL LOADED TOPICAL GEL (GB3)

TABLE 14: STABILITY STUDY OF GB 3 AT 40°C

Time (Months)	Drug content (mg)	% Remained	Log % Remained	%DC
0	10	100	2	100
1 month	9.59	90.41	1.9562	95.9
2 month	9.56	90.44	1.9563	95.6
3 month	9.44	90.56	1.9569	94.4

From the stability studies, it was concluded that the optimized co-crystal loaded gel batch has stability in human skin. There was no change in colour and viscosity was observed. Hence the formulated gel batch 3 was indicated stable.

CONCLUSION: In the current research article, we have fabricated three pharmaceutical co-crystals with using different co-formers such as oxalic acid, succinic acid and L-proline. In between the formulated co-crystals Hydrogen bonds formed

between ECZN and co-formers and enhancement in aqueous solubility was determined by performing in-vitro dissolution study. ECZN: Oxalic acid co-crystal showed better aqueous solubility as compared to ECZN: succinic acid co-crystal and ECZN: L-proline co-crystal. For administration, ECZN was incorporated into the topical gel. A simple, precise method was established for the evaluation of different parameters of topical gel such as pH, viscosity, Spreadability. To check skin permeability of gel batches, *ex-vivo* study on goat skin was conducted. Gel batch 3 showed optimum permeability as compared to other batches. Optimized ECZN co-crystal loaded topical gel showed better antifungal activity as compared to pure ECZN. From the above results it was concluded that the formulated ECZN co-crystal succeeded to enhanced aqueous solubility of pure ECZN, formulated gel showed better antifungal effect against candida species and showed more stability at 25°C as compared to 40°C.

ACKNOWLEDGEMENTS: Nil

CONFLICTS OF INTEREST: Nil

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