

Original Article

## BIOAVAILABILITY ENHANCEMENT OF POORLY SOLUBLE RALOXIFENE BY DESIGNING INCLUSION COMPLEX WITH $\beta$ -CYCLODEXTRIN

NIRMAL SHAH\*, A K SETH, R. BALARAMAN

Department of Pharmacy, Sumandeep Vidyapeeth, Vadodra, Gujarat 391760, India  
Email: nimspharma@gmail.com

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### ABSTRACT

**Objective:** Raloxifene hydrochloride (RLX) is widely used in the treatment of osteoporosis, but due to its extensive first pass metabolism bioavailability of RLX is remaining only 2%. In addition of that being from BCS class II, RLX has poor water solubility. Therefore the objective of present research work was to enhance solubility and dissolution rate of RLX by formulating inclusion complex with  $\beta$  cyclodextrin ( $\beta$ -CD) as a carrier.

**Methods:** Inclusion complex was prepared by various methods like physical mixture, co-precipitation method and kneading method using different drug to carrier ratios (1:1, 1:2 and 1:3).

**Results:** Inclusion complex prepared with co-precipitation method had shown 5.5 fold improvements in water solubility and significant increment in dissolution rate when compared with plain RLX. Optimized formulation was characterized by Fourier transform infrared spectroscopy, Differential scanning calorimetry, X-ray diffraction and Scanning electron microscopy studies for their compatibility, thermal analysis, crystallinity and surface morphology determination, respectively. Results of DSC and XRD study suggested the conversion of RLX from crystalline form to amorphous form. Stability studies showed stable formulation up to the period of 6 months. *In vivo* pharmacokinetic study was also conducted in wistar rats for optimized drug loaded inclusion complex that showed nearby two fold increments in drug bioavailability compared to plain drug suspension.

**Conclusion:** From these studies, it can be concluded that solubility and dissolution rate of poorly soluble raloxifene could be remarkably increased by formulating into the inclusion complex with  $\beta$ -CD which results in significant improvement in bioavailability of poorly soluble RLX.

**Keywords:** Bioavailability, Dissolution rate, BCS classification, Inclusion complex, Carrier.

### INTRODUCTION

Poor water solubility has been recognized to almost half of the 150,000 new molecular entities (NMEs), synthesized annually by pharmaceutical companies, and is also claimed to reduce the performance of more than 10% of successfully marketed drugs.

Up to 40% of lipophilic drug candidates may fall short to reach market *al. though* exhibiting potential pharmacodynamic activities. Meanwhile, some lipophilic drugs on the market have to be administered at high doses. Therefore, various formulation strategies have been investigated to improve solubility and rate of dissolution followed by oral bioavailability of lipophilic drugs [1].

Osteoporotic fractures lead to morbidity and mortality. The occurrence of osteoporosis and the associated economic burden will rise as the population ages. Several antiresorptive agents currently available for prevention or treatment of postmenopausal osteoporosis include the bisphosphonates (Alendronate, Risedronate, and Ibandronate), calcitonin, estrogen, and the selective estrogen receptor modulator raloxifene (RLX) [2]. In the Multiple Outcomes of Raloxifene Evaluation (MORE) study, raloxifene 60 mg/day appreciably decreased the risk of new vertebral fractures by 30% and 55% compared with placebo, in women with and without prevalent vertebral fractures, respectively [3, 4].

Raloxifene is a selective estrogen receptor modulator (SERM) with a proven estrogen agonist action on bone that leads to an improvement in bone mass density, a reduction in bone turnover [3] and a decrease in vertebral fractures in post-menopausal women with osteoporosis [5]. Although it belongs to class II drug according to biopharmaceutical classification system (BCS), it has only 2% bioavailability. Hence it is essential to increase solubility and dissolution rate of raloxifene which may lead to enhancement in bioavailability through oral dosage form [6].

In the present study, inclusion complex was formulated to enhance solubility of drug by complexing with  $\beta$ -cyclodextrin ( $\beta$ -CD).

Cyclodextrins (CDs), with lipophilic interior cavities and hydrophilic outer surfaces, are capable of interacting with a large variety of guest molecules to form noncovalent inclusion complexes.  $\beta$ -CD has been widely used in the early stages of pharmaceutical applications for the reason of its ready availability and cavity size suitable for the widest range of drugs [7].

In the present research work, inclusion complex of RLX with  $\beta$ -CD was prepared to study the effect of different methods (physical, co-precipitation and kneading method) on drug's aqueous solubility. The prepared inclusion complexes were studied for *in vitro* release, Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), X-ray diffractometry (XRD) and scanning electron microscopy (SEM). Bioavailability enhancement in selected inclusion complex (optimized based on its improved solubility and dissolution rate) was checked by performing *In vivo* study for the determination of various pharmacokinetic parameters. The objective of this work was to improve solubility and dissolution rate followed by bioavailability of poorly soluble RLX by complexing drug with  $\beta$ -CD.

### MATERIALS AND METHODS

#### Materials

RLX was obtained as gift sample from Aarti drugs Pvt Ltd, Mumbai, India.  $\beta$ -CD was purchased from Merck specialities Pvt Ltd, Mumbai. All other reagents used in work were of analytical grade.

#### Formulation of inclusion complex of RLX with $\beta$ -CD

The inclusive complex of RLX with  $\beta$ -CD was prepared by following methods. Formulation codes for all methods are given in table 1. Different ratios of RLX and  $\beta$ -CD (1:1, 1:2 & 1:3) were taken for the preparation of inclusion complexes for all methods.

#### Physical mixture

RLX with  $\beta$ -CD were mixed in a mortar and pestle for 1h with continuous trituration. Mixture was passed through sieve no. 80 and stored in desiccators over fused calcium chloride.

**Table 1: Formulation codes for inclusion complex of RLX &  $\beta$ -CD**

Formulation codes	Method of preparation	Drug: carrier ratio
IC-1	Physical method	1:1
IC-2		1:2
IC-3		1:3
IC-4	Co-precipitation method	1:1
IC-5		1:2
IC-6		1:3
IC-7	Kneading method	1:1
IC-8		1:2
IC-9		1:3

### Co-precipitation method

Accurately weigh quantity of drug and  $\beta$ -CD were dissolved in methanol and distilled water, respectively. Drug solution was added slowly to  $\beta$ -CD solution and mixture was stirred at room temperature for 1h followed by slowly evaporation on a boiling water bath for complete evaporation of solvents. The inclusion complex precipitated as a crystalline powder which was pulverized and passed through sieve no. 80 and stored in a desiccator having fused calcium chloride [8, 9].

### Kneading method

Accurately weigh quantity of  $\beta$ -CD was triturated in the mortar containing little quantity of 50% methanol to get slurry like consistency. Drug was incorporated into the slurry and triturated further for 1h. Mixture was air dried at 25 °C for 24 h, pulverized and passed through sieve no. 80 and stored in desiccators over fused calcium chloride [8, 9].

### Characterization of inclusion complex

#### Percentage yield

The yield was calculated by dividing the weight of the collected inclusion complex with the weight of all the non-volatile components used for preparation of the inclusion complex.

$$\text{Percentage yield} = \frac{\text{Weight of inclusion complex recovered}}{\text{Theoretical weight (drug+carrier)}} \times 100$$

#### Drug content

Accurately weigh quantity of the inclusion complex (equivalent to 5 mg of RLX) was transferred to 50 ml volumetric flask. Volume was made up to the mark with buffer system (citro phosphate buffer pH 7.6 containing 1% polysorbate 80). The absorbance of the solution was measured after suitable dilution at 288 nm against buffer system as blank using UV-visible spectrophotometer (UV-1800, Shimadzu, Japan).

#### Solubility study in water

Excess amount of inclusion complex was added to 10 ml water in stopper volumetric flask followed by sonication for 10 min. Flasks were kept at 25±2.0°C in an isothermal orbital shaker (MSW-132, MAC scientific Works Pvt Ltd, Delhi) for 24 h to reach equilibrium. The equilibrated samples were removed from shaker and centrifuged at 5000 rpm for 15 min using remi centrifuge (C-24 BL, Remi instruments Pvt Ltd, Mumbai) [10]. The water solubility profile of inclusion complex was determined by analyzing the supernatant spectroscopically at 288 nm.

#### Drug excipients compatibility studies of optimized batch

Drug excipients compatibility study was carried out by FTIR (IRAffinity-1, Shimadzu, Japan) for pure drug,  $\beta$ -CD, physical mixture and optimized inclusion complex to study interaction between drug and excipients. Sample was mixed with KBr in a ratio of 1:300 and FTIR spectrum was recorded in the range of 4000–400 cm<sup>-1</sup> using FTIR spectrophotometer [8-12].

#### In vitro drug release

Drug release from plain RLX suspension and optimized inclusion complex were determined in 900 ml citro phosphate buffer pH 7.6

containing 1% of polysorbate 80 at 37±0.5 °C with a stirrer rotation speed of 75 rpm using USP dissolution test apparatus type II (TDT-08L, Electrolab, Mumbai). RLX- $\beta$ -CD inclusion complex was taken equivalent to 60 mg of RLX in a capsule shell [6]. Aliquots of 5 ml sample were withdrawn at predefined time interval with a pipette and replaced with fresh buffer at each time [13]. The samples were filtered through cellulose acetate membrane filter (0.45 $\mu$ m) and analyzed by using UV-Visible spectrophotometer at 288 nm. Each dissolution test was carried out in triplicate.

#### Differential scanning calorimetry

DSC is widely used in thermal analysis to observe endothermic and exothermic reactions. It could be very useful in preformulation studies as it gives information about possible drug-excipients interactions in formulation. Thermograms of RLX,  $\beta$ -CD & optimized inclusion complex were obtained by using differential scanning calorimeter (DSC TA-60, Shimadzu, Japan). Samples were weighed directly in aluminum pan and scanned at 50-300 °C temperature under dry nitrogen atmosphere. Heating rate of 10 °C/min was used [14].

#### X-Ray diffraction studies

XRD study can become useful tool to find out amorphous or crystalline form of formulations [13]. XRD study of RLX,  $\beta$ -CD, physical mixture & optimized inclusion complex was carried out by X Ray Diffractometer (Xpert Pro MPD, Panalytical, The Netherlands) where CuK $\alpha$  radiation wavelength of 1.5405 Å was used as X-ray source. For the study, samples were placed in the glass sample holders and scanned from 2 ° to 60 ° at a scan angular speed (2 $\theta$ /min) of 2 °/min with 40kv operating voltage and 30 mA current.

#### Surface morphology study

The surface morphology and shape of solid particles can be studied by SEM [15]. Surface morphology of plain RLX and optimized inclusion complex were obtained by scanning electron microscope (JEOL JSM-5610LV, England). Prior to examination, the samples were mounted on to metal stubs using a double-sided adhesive tape under vacuum. Images were observed at various magnifications, operated with an acceleration voltage of 15 kV and working distance of 20  $\mu$ m was maintained [16, 17].

#### Stability study

The selected formulation was subjected to stability studies as per ICH guidelines [18]. The samples were placed in vials and kept at 25°C ± 2 °C/ 60% ± 5% RH ± SD and 40°C ± 2°C/ 75% ± 5% RH [16] using stability chamber (Macro scientific work Pvt Ltd, Delhi) over period of six months. The samples were analyzed for physical appearance and the drug content at the interval of 0, 15, 30, 60, 120 & 180 days.

#### In vivo Pharmacokinetic study in Wistar rats

In vivo pharmacokinetic study was carried out for optimized formulations IC-6 and plain drug suspension to check the bioavailability of RLX, as per the protocol discussed underneath.

#### Experimental animals

The experimental protocol in the present study was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Institutional Animal Ethics Committee (IAEC). Healthy female Wistar rats weighing 200-250g were used for the study [14]. Rats were housed in polypropylene cages, maintained under standardized condition (12 h light/dark cycle, 24 °C, 35-60 % humidity) and allowed free access to diet (Nav Maharashtra oil mills ltd, Pune) and purified drinking water [19, 20].

#### Bioanalytical method

In the present research work, HPLC (UFLC, Shimadzu corporation, Japan) prominence liquid chromatographic system was used for chromatographic separation which is controlled by LC solution software (Version 1.24 Sp1, Shimadzu corporation, Japan). The system was equipped with Binary pump (LC 20AD version 1.10, Shimadzu corporation, Japan), a manual injector, a column (C18 250 mm × 4.6 mm, 5  $\mu$ m) (Luna, Phenominax, USA) and a photo diode

array (PDA) detector (SPD 20A version 1.08, Shimadzu corporation, Japan). The mobile phase used for separation is 67% 0.05M ammonium acetate buffer (pH was adjusted to 4.0 with glacial acetic acid) and 33% acetonitrile [14, 19, 21] which was delivered isocratically with a flow rate of 1 ml min<sup>-1</sup>. The injection volume used was 20 µL and the analysis was carried out at wavelength of 288 nm [19, 22].

### Standard sample treatment procedure

#### Preparation of standard solution

A series of standard solutions of RLX were prepared in methanol, in the range of 0.1-10 µg/ml. Samples were prepared by addition of standard solution of drug in methanol (50 µl) with acetonitrile (200 µl) to eppendorf tube containing blank plasma (100 µl) [20]. This prepared mixture was treated as per the method given in sample extraction procedure. Final RLX concentrations in plasma were obtained 0.05-5 µg/ml.

#### Sample extraction procedure

Standard solution of drug in methanol and acetonitrile were added to the eppendorf tube containing plasma sample (volume as mentioned above) followed by meticulously vortex mixing (Macro Scientific Work Pvt Ltd, Delhi) for 30 sec. Centrifugation was carried out for the separation of denatured protein at 15,000 rpm for 10 min at -6 °C [19,20]. After centrifugation, aliquots of 20 µl of the filtered supernatant (0.45µm membrane filter) were injected into HPLC system and drug content was measured at 288 nm for analysis.

#### Experimental design

The animals were fasted at least 12 h prior to dose administrations and for 4 h after dosing with free access to water. Animals were divided into two groups each consisting of six animals. All animals were given different formulations group wise as described underneath [22].

Group I: Control group (Plain RLX suspension in 0.5% w/v sodium CMC, 15 mg/kg, p. o.)

Group II: Formulation (optimized batch IC-6, 15 mg/kg, p. o.)

A glass capillary was inserted into retro orbital plexus under mild ether anesthesia for the withdrawn of blood samples (0.5 ml) at a

time interval of predose, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h post dose. Blood samples were collected in micro centrifuge tubes containing anticoagulant. The plasma was separated immediately from aforementioned samples after centrifugation at 5,000 rpm at 4 °C for 10 min and stored immediately at -20 °C until further analysis [19,22]. Samples were analyzed by standard HPLC method after standard sample extraction procedure.

#### Pharmacokinetic data analysis

PK solver add-in program for Microsoft Excel (version 1.0, China) was used for the estimation of Pharmacokinetic parameters. Various parameters like maximum plasma concentration (C<sub>max</sub>), time for achieving maximum plasma concentration (T<sub>max</sub>), area under curve [AUC]<sub>0-24</sub> and relative bioavailability (F) were determined. Each experiment was carried out in triplicate.

#### Statistical analysis

The obtained data was statistical analyzed by one way analysis of variance (ANOVA) using student's *t*-test. Graph Pad Instat program version 3.01 (Graph Pad Software, Inc. CA, USA) was utilized to determine the significance difference between formulations. The level of statistically significance was selected as less than *p*<0.05.

## RESULTS AND DISCUSSION

### Characterization of inclusion complex

#### Percentage yield, drug content and water solubility

Percentage yield, drug content and water solubility of prepared inclusion complexes are shown in table 2.

Percentage yield of all batches were calculated and it was found that there was no significant difference in percentage yield of all nine batches. But it was also observed that first three batches of physical mixture showed more yield compared to other two methods. The fact for this may be easiness of physical mixture method to make inclusion complex where in co-precipitation method and kneading method, there might be chance of losing the product due to solidification of mixture.

**Table 2: Percentage yield, drug content and water solubility of all prepared batches**

Formulation codes	Percentage yield (%)	Drug content (%)	Water solubility (mg/ml)
IC-1	99.08±0.82	48.05±1.34	0.17±0.02
IC-2	98.87±0.98	32.10±0.75	0.22±0.02
IC-3	98.95±0.87	24.13±0.54	0.29±0.03
IC-4	96.33±1.76	47.19±0.91	0.39±0.02
IC-5	96.12±1.38	32.02±1.18	0.50±0.05
IC-6	96.41±2.05	24.11±0.53	0.61±0.04*
IC-7	97.61±0.72	46.74±1.08	0.28±0.03
IC-8	97.24±1.03	31.62±1.12	0.41±0.04
IC-9	96.68±1.14	23.88±0.24	0.48±0.02
Plain drug solubility (mg/ml)			0.11±0.02

Value are expressed as mean±SD; n=3 (\* *p*<0.05)

There was no significant effect of different methods of preparation for inclusion complexes on drug content, but from the study it was noted that for a same method if ratio of drug to carrier is increased then there is increment in drug content which support the fact that more amount of carrier may accommodate larger amount of drug in a complex form.

From the solubility study, it was observed that plain drug has 0.11±0.02 mg/ml solubility in water. Water solubility is drastically increased in a range from 0.17±0.02 to 0.61±0.04 when plain drug was converted into complex form with β-CD. It was also noted that solubility of drug in physical method was significantly less compared with other two methods. Maximum drug solubility was found in batch IC-6 prepared by co-precipitation method with drug: carrier ratio 1:3, which was found to be 5.5 fold more than plain drug that showed significant difference in comparison with plain drug

suspension (*p*<0.05). Therefore based on solubility study batch IC-6 was selected for further study.

#### Drug excipients compatibility study

Drug excipients compatibility study was carried out by FTIR for optimized inclusion complex IC-6 as shown in fig. 1. From the compatibility study it was observed that characteristic peaks of drug i.e. 945.12 cm<sup>-1</sup> (Benzene ring), 1456.26 cm<sup>-1</sup> (-S-benzothiophene), 1600.92 cm<sup>-1</sup> (-C-O-C-stretching) and 1647.21 cm<sup>-1</sup> (C=O stretching) in inclusion complex IC-6 was found to be nearby similar with plain drug spectra. Thus, it was revealed that there was no significant physiochemical interaction between drug and β-CD was found when formulating inclusion complex. This result indicates compatibility between drug and carrier. From the result, it was found that pronounced enhancement in dissolution rate up to 93.46±0.26 % is

shown by RLX:  $\beta$ -CD inclusion complex compared to that of  $50.39 \pm 0.39$  % by pure drug after 150 min. This is because of higher hydrophilicity and wetting property of  $\beta$ -CD. The results of the statistical analysis indicated significant dissolution rate enhancement of RLX from RLX:  $\beta$ -CD inclusion complex ( $p < 0.05$ ) compared with pure RLX. Optimized inclusion complex IC-6 also showed significant enhancement ( $p < 0.05$ ) in dissolution rate compared to previously carried out study by Patil PH *et al.* ( $84.47 \pm 0.84$  % drug release from RLX: Hydroxypropyl  $\beta$ -CD inclusion complex) [6].

Thermograms of RLX and  $\beta$ -CD showed endothermic peaks at  $272.92^\circ\text{C}$  and  $117.79^\circ\text{C}$  corresponding to their melting points, respectively. Thermogram of optimized inclusion complex showed the presence of  $\beta$ -CD peak in hydrated form at  $96.21^\circ\text{C}$  whereas broad peak of drug was observed at  $273.30^\circ\text{C}$  suggesting the conversion of RLX from crystalline to amorphous form or solubilization of drug into melted carrier.

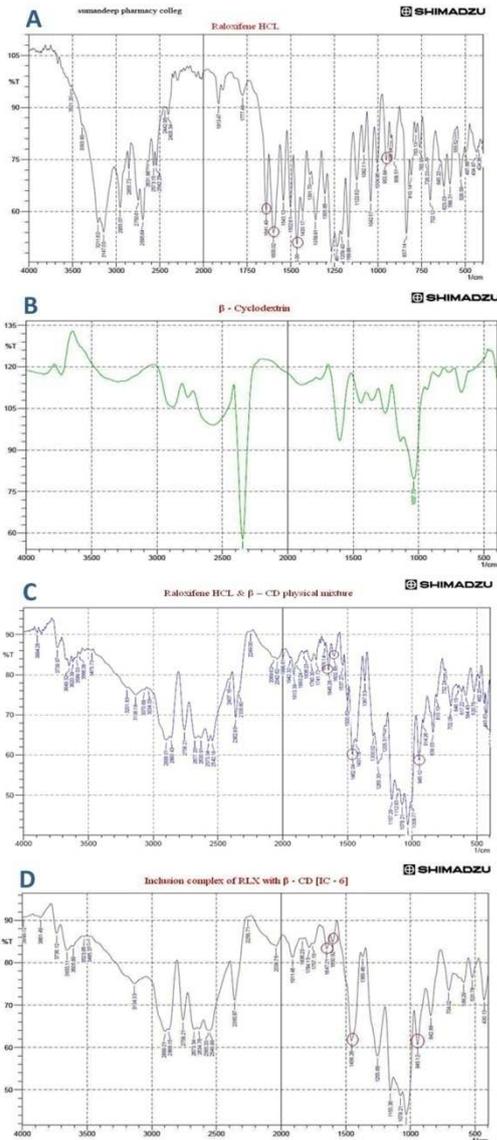


Fig. 1: FTIR spectra of (A) Raloxifene HCL, (B)  $\beta$ -cyclodextrin, (C) Physical mixture and (D) optimized inclusion complex of RLX with  $\beta$ -CD (IC-6)

**In vitro drug release**

*In vitro* drug release study was carried out between plain drug and optimized inclusion complex IC-6 as shown in fig. 2.

**Differential scanning calorimetry**

DSC measurements offer a close look at the crystallization and thermal behaviour of the inclusion complex formulations. The DSC thermograms of RLX,  $\beta$ -CD and inclusion complex of RLX with  $\beta$ -CD are shown in fig. 3.

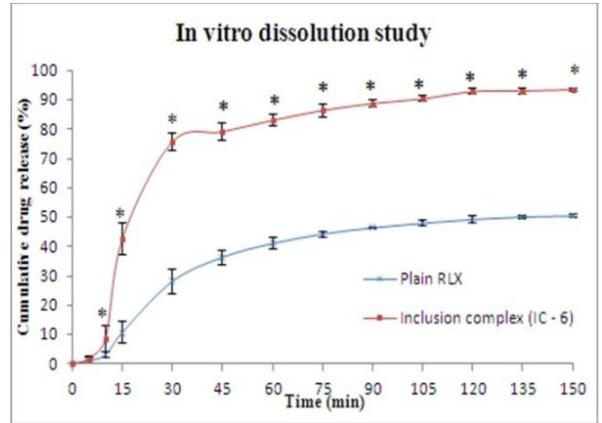


Fig. 2: Dissolution profile of plain RLX suspension & inclusion complex (IC-6), \*  $p < 0.05$

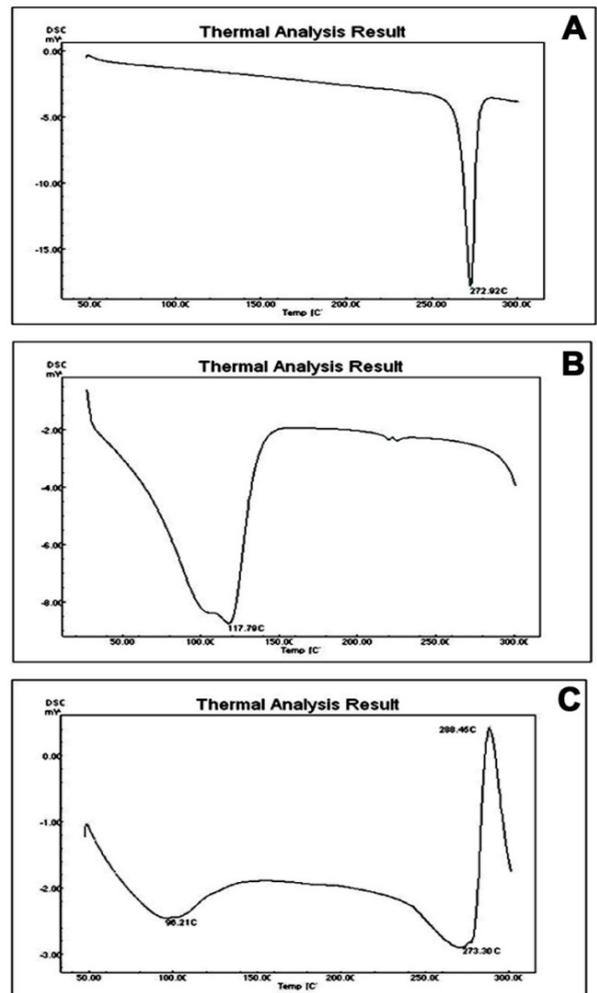


Fig. 3: DSC Thermogram of (A) Raloxifene HCL, (B)  $\beta$ -cyclodextrin and (C) Optimized inclusion complex (IC-6)

### X-Ray diffraction studies

XRD studies were performed in conjugation with DSC to verify the reduction of crystallinity of RLX. As shown in fig. 4, the X ray diffraction patterns were recorded for pure drug,  $\beta$ -CD, physical mixture and inclusion complex of RLX with  $\beta$ -CD.

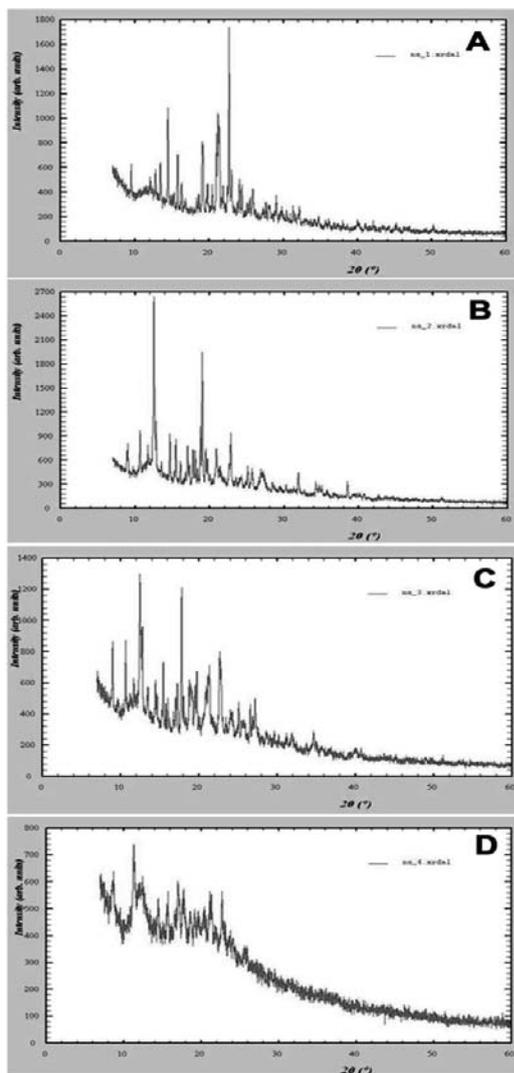


Fig. 4: XRD patterns of (A) Raloxifene HCL, (B)  $\beta$ -cyclodextrin (C) Physical mixture and (D) Optimized inclusion complex (IC-6)

Diffraction spectrum of drug and physical mixture of RLX &  $\beta$ -CD showed distinct peaks at  $2\theta$  scale, which was indicating the crystalline nature of drug. In case of optimized inclusion complex XRD pattern, there was a considerable reduction in relative integrated intensity of all peaks and no distinct peak of drug was observed throughout the  $2\theta$  scale.

This finding was supported with results obtained from DSC studies. Therefore it can be concluded that RLX drug is completely converted into amorphous state when formulated in inclusion complex with  $\beta$ -CD that contribute enhancement in dissolution rate of RLX from inclusion complex.

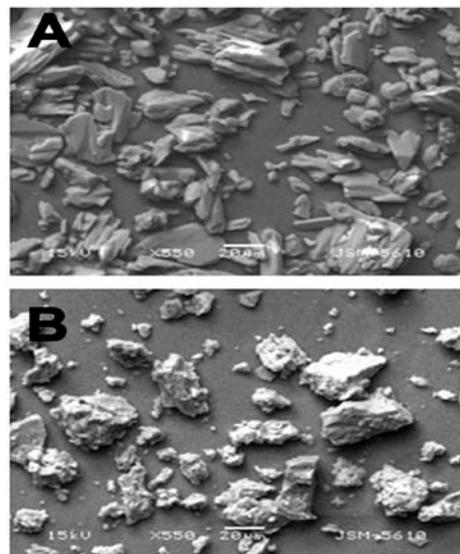


Fig. 5: SEM image of (A) Raloxifene HCL and (B) Optimized inclusion complex (IC-6)

### Surface morphology study

As shown in fig. 5, scanning electron microscopic photograph of plain drug showed longer crystal with specific morphology, whereas for optimized RLX and  $\beta$ -CD inclusion complex a decrease in crystallinity was observed. This confirms complexation of drug with carrier  $\beta$ -CD attributed due to the conversion of amorphous form of drug in inclusion complex.

### Stability study

As shown in table 3, stability data showed insignificant change in physical appearance and drug content of formulation in both stability conditions compared with initial observation of same batch. Thus, it was revealed that there is no significant physiochemical interaction between drug and excipients even after six months of storage period which indicate compatibility between them.

### In vivo pharmacokinetic study

RLX being a BCS-II drug having very less aqueous solubility and also has extensive first pass metabolism, which results in a low oral bioavailability of drug. Therefore, a trial was made to increase solubility followed by bioavailability using concept of complexation method.

In the present work, plain drug suspension and optimized batch IC-6 were administered orally to female Wistar rats followed by determination of various pharmacokinetics parameters. Pharmacokinetic parameters of formulations was calculated and shown in table 4.

Table 3: Stability study data for optimized inclusion complex (IC-6)

Time (days)	25°C±2°C/60%±5% RH		40°C±2°C/75%±5% RH	
	Physical appearance	Drug content	Physical appearance	Drug content
0	Yellow free flowing Powder	24.11±0.53	Yellow free flowing Powder	24.11±0.53
15		24.10±0.12		24.10±0.37
30		24.08±0.75		24.12±0.52
60		24.09±0.44		24.06±0.21
120		24.05±0.36		23.98±0.11
180		24.00±0.33		23.95±0.17

Value are expressed as mean±SD; n=3

Table 4: Comparative study of the pharmacokinetic parameters of optimized batch IC-6 and plain drug suspension

Sample	Pharmacokinetic parameters				
	C <sub>max</sub> ± SD (ng/ml)	T <sub>max</sub> ±SD (h)	[AUC] <sub>0-24</sub> ±SD (ng*h/ml)	t <sub>1/2</sub> (h)±SD	F
Plain RLX suspension	39.52±4.44	8	452.46±25.65	7.61±0.34	--
IC-6	83.49±4.35*	6	878.64±39.22*	8.75±0.14	1.94

Value are expressed as mean±SD; n=3, F-Relative bioavailability, \*p<0.05 compared with plain RLX suspension

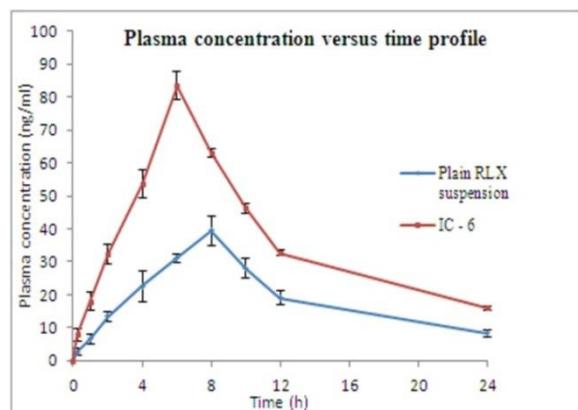


Fig. 6: Plasma concentration versus time profile of the optimized IC-6 and plain drug suspension

Fig. 6 showed higher C<sub>max</sub> for optimized batch IC-6 (83.49±4.35 ng/ml) at 4 h with respect to plain drug suspension (39.52±4.44 ng/ml) at 8 h. As shown in table 4, the [AUC]<sub>0-24</sub> of IC-6 was found 878.64±39.22ng\*h/ml in comparison of plain drug suspension 452.46±25.65 ng\*h/ml and was found statistically significance (p<0.05). This result may be attributed due to remarkable improvement in solubility and dissolution rate of optimized inclusion complexes than the plain drug. Elimination half-life (t<sub>1/2</sub>) of inclusion complex was also found higher than drug suspension. All these result showed nearby two fold increments in bioavailability of drug in case of drug loaded inclusion complex which confer its potential for improved oral delivery of RLX.

## CONCLUSION

Co-precipitation method was successfully employed to prepare stable RLX and β-CD inclusion complex. Lipophilic inner cavities and hydrophilic outer surfaces of cyclodextrins are able for interactions with a huge range of guest molecules to form non covalent inclusion complexes with drug molecules. Compared to plain drug, inclusion complex had shown 5.5 fold improvements in water solubility and significant enhancement in dissolution rate of drug. In complex formation, drug was converted into amorphous form which was confirmed by XRD and DSC studies. *In vivo* pharmacokinetic study of optimized inclusion complex showed nearby two fold increments in bioavailability of drug compared to plain drug suspension. Therefore, it can be concluded that even at large scale production, inclusion complex can be potentially exploited in improving water solubility and dissolution rate followed by bioavailability of poorly soluble raloxifene.

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## CONFLICT OF INTERESTS

Declared None

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