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Formulation, Development, Evaluation and Solubility Enhancement of Lercanidipine Hydrochloride by Inclusion Complex Method.

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ABSTRACT

Background: Cyclodextrins (CDs) are a group of cyclic oligosaccharides compounds made up of glucopyranose units which are linked by (α-1, 4) linkage and obtained after enzymatic action on starch molecule.

Aims and objective: The objective of present study is to study the interaction of LER (LER) and Cyclodextrins (CDs) in terms of formation on inclusion complex.

Material and methods: The formation and conformation of the inclusion complex in the optimized inclusion complex was studied in detail by fourier-transform infrared spectroscopy (FT- IR), powder X-ray diffractometry (PXRD) and Proton nuclear magnetic resonance (NMR).

Result: Significant increase in the DE_{30} and MDT was obtained with the optimized inclusion complex. Result of FTIR, PXRD, ^{1}H NMR and DSC confirms that strong interaction takes place between LER and HPβCD.

Conclusion: Physical mixing , kneading and freeze drying technique, freeze drying method is most successful in producing inclusion complex with faster and higher dissolution.

Keywords: Cyclodextrins, Inclusion ,Complexation,Lercanidipine.

INTRODUCTION

Cyclodextrins (CDs) are a group of cyclic oligosaccharides compounds made up of glucopyranose units which are linked by (α-1, 4) linkage and obtained after enzymatic action on starch molecule. Cyclodextrins are having unique molecular structure in which they are having skeletal carbons and ethereal oxygens of glucose reside in the inner side [1-3].This characteristic structure produces a lipophilic environment and exterior structure of Cyclodextrin is made up of hydroxyl groups which confer hydrophilicity. Hence central cavity provides a hydrophobic environment in which a drug molecule can enter to form complex and hydrophilic exterior which impart water solubility to the complex formed [4]. Structurally cyclodexrin exists in a cone shape having narrow and wide rims, this cavity is a structure in which guest molecules/drug enters and forms inclusion complex with non-covalent interactions such as hydrophobic interaction, electronic effects, Van Der Waals forces and steric factors [5-7]. Effective Inclusion Complex formation between Cyclodextrin and guest/drug molecule alters the physicochemical properties such as stability, solubility, dissolution rate and pharmacodynamics properties such as drug bioavailability of guest/drug molecules [8-10]. Inclusion complexes can be formed using various techniques such as physical mixing, kneading, spray drying, freeze drying, co precipitation and solvent evaporation methods [11, 12]. Method of preparation of inclusion complex has a prominent effect on efficiency of complexion and thus on the effect of the drug in complex form [13]. As such there is no any ideal method available to form CD- Drug inclusion complexes, however based on the guest and host characteristics, suitable method and best experimental conditions can be identified in order to achieve the goal of Cyclodextrin complexation[14].

LER being BCS Class II drug and having only 10% or oral bioavailability is a good candidate to form inclusion complex with Cyclodextrin [15, 16].

AIM AND OBJECTIVE

The objective of present study is to study the interaction of LER (LER) and Cyclodextrins (CDs) in terms of formation on inclusion complex. From the various CDs derivatives, β Cyclodextrin (βCD) and Hydroxy Propyl β Cyclodextrin (HPβCD) were selected CDs for formation of inclusion complex. The LER/ CDs (βCD and HPβCD) inclusion complexes were prepared by two different methods namely kneading method and freeze drying method. Phase solubility was carried out to calculate stability constant and to know the inclusion stoichiometry of complexes. Saturation solubility and dissolution profile was obtained for all products and based on the results obtained, optimized inclusion complex was identified. The formation and conformation of the inclusion complex in the optimized inclusion complex was studied in detail by fourier-transform infrared spectroscopy (FT- IR), powder X-ray diffractometry (PXRD) and Proton nuclear magnetic resonance (NMR). Results obtained from this study showed promising approach to obtain the LER inclusion complex with high water solubility, higher dissolution properties and in turn better bioavailability.

MATERIAL AND METHODS

Preparation of Inclusion Complexes

Inclusion complexes of LER with CDs were prepared in different molar ratio of drug to CDs. The molar ratios were decided on the basis of phase solubility studies. Two methods namely kneading and freeze drying were used to prepare inclusion complexes with different molar ratios (Table 1)

Physical Mixture

To prepare a physical mixture, LER and CDs were weighed accurately, sieved through 65# sieve and mixed uniformly by adding LER slowly into CDs in a mortar with slow but continuous trituration. Resulting mass was then passed through 65 # sieve and stored in an amber colored vial [4, 19].

Kneading Method

The amounts of CDs and LER for complex preparation were calculated on molar ratio basis. Accurately weighed amount of βCD and LER were transferred to a glass mortar pestle followed by trituration with small volume of ethanol-water (1:1 v/v). Resulted slurry was kneaded uniformly for 45 minutes. Obtained paste was dried under vacuum at room temperature Dried mass thus obtained was grounded in mortar, passed through sieve no. 100 and stored in amber coloured vials for further characterization.

HPβCD complex of LER were prepared by transferring accurately weighed amount of LER and HPβCD to mortar with few mL of ethanol. Resulting mixture was kneaded for 60 min. After trituration, obtained mass was stored in a vacuum desiccator for 48 h at room temperature. The dried mass was weighed, sieved through sieve no. 100 and stored in desiccator in amber colored vials for further characterization[20].

Freeze Drying Method

Calculated amounts of CDs and LER were accurately weighed dissolved in water and ethanol respectively. Resulting solutions were mixed and magnetically stirred for 10 hours at RT protected from light. Obtained solution was filtered through 0.45 µ membrane filter and stored at - 20° C followed freeze drying in a freeze dryer for 24 hours [21].

Evaluation of Inclusion Complexes

Determination of LER in Inclusion Complexes

Physical mixture and inclusion complexes equivalent to 10 mg of LER was calculated.

Saturation Solubility studies

***In vitro* dissolution studies**

Drug release behaviour from physical mixtures and inclusion complexes was studied by *in vitro* dissolution performance. Dissolution of LER pure, physical mixtures and inclusion complexes were carried out in USP dissolution apparatus type II using 900 mL of 0.1 N HCl (pH 1.2) as a dissolution medium at 100 rpm and 37±0.5°C for 1 hour. Accurately weighed formulation equivalent to 10 mg of LER was weighed, filled in capsule and was introduced into dissolution vessel. At predetermined interval, 10 mL aliquots of dissolution medium was withdrawn followed by addition of same amount of fresh dissolution medium for replacement. Withdrawn samples were filtered through whatman filter paper no. 41 and amount of LER was measured spectrophotometrically at 236 nm using 0.1 N HCl as a blank in double beam UV Vis Spectrophotometer. The dissolution studies were carried out in triplicate and % cumulative drug release was plotted against time to obtain dissolution profiles of LER pure, physical mixtures and inclusion complexes.

Solid State Characterization of Optimized inclusion complex

Inclusion complexes of βCD and HPβCD having maximum saturation solubility and/or *in vitro* dissolution was selected and further subjected to solid state characterization.

Fourier Transform Infrared Spectroscopy

The FTIR spectra of LER, pure βCD, pure HP βCD, Physical mixtures and optimized inclusion complexes were recorded.

Differential Scanning Calorimetry (DSC)

The DSC thermogram of LER, pure βCD, pure HP βCD, Physical mixtures and optimized inclusion complexes were recorded.

X-ray diffraction

The Powdered X-ray Diffraction of LER, pure βCD, pure HP βCD, Physical mixtures and optimized inclusion complexes were recorded.

Nuclear Magnetic Resonance Spectrometry

The most accurate formation of an inclusion complex of LER with βCD was studied through NMR Spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded using 400 MHz NMR Spectrometer. The spectrometer was equipped with a 5 mm multinuclear direct detection BBO probe with z-gradient. LER pure, βCD, physical mixture and inclusion complex were subjected to NMR spectroscopy using DMSO as solvent and chemical shifts were reported as ppm[22].

Stability and Photostability Study

Stability study of optimized inclusion complex was performed as per the procedure

RESULT & DISCUSSION

Preliminary Study

Phase Solubility Study

The Phase solubility diagram of LER in βCD and in HPβCD at 25ºC is depicted in Fig. 1 The phase diagram obtained is classified as type $A_L$type of linear host-guest relationship and indicates linear increase in apparent solubility of LER with increase in concentration of CDs. Assuming a 1:1 stoichiometry, the value of apparent stability constant Ks was found to be 428.18 M^{-1} for βCD and 798.571 M^{-1} for HPβCD (Table 2). The ideal value of a stability constant lies within 100 and 1,000 M^{-1}, obtained value indicates that desirable interaction will take place between LER and CDs [18, 23]. Amongst βCD and HPβCD, ability to form inclusion complex of later is slightly more as stability constant of βCD is 1.86 fold smaller than that of HPβCD. Based on the result obtained of phase solubility study, interaction of LER for both βCD and HPβCD could be achieved and so inclusion complex for both the cyclodextrins were prepared and characterized.

Gibbs-free energy ($\Delta G°_{tr}$) study

Gibb's free energy was calculated to know the process of transfer of LER from pure water to solution of CDs [24].The result of Gibb's free energy of transfer of LER from aqueous solution to cavity of βCD and HPβCD is shown in Table 3 For both cyclodextrins value of $\Delta G°_{tr}$ is negative which suggests that favourable interaction can take place between LER and both cyclodextrins. Moreover the value of $\Delta G°_{tr}$ decreases with increase in concentration of cyclodextrin demonstrates that reaction becomes more favourable as the concentration of cyclodextrin is increased. This thermodynamic parameter gives strong evidence about formation of inclusion complex molecules. Many scientific works have reported similar findings [25-27] with different values of $\Delta G°_{tr}$ because of specific guest host interactions. Variation in equilibrium between free and complexed states gives rise to different values of $\Delta G°_{tr.}$ Relative affinities of the drug for the cavity of CD and its concentration affects the rate of release of drug from inclusion complex.

Preparation and Evaluation of Inclusion Complex of LER

Determination of LER in Inclusion Complexes

Result for the content determination in inclusion complexes is shown in Table 4.

The content of inclusion complexes varies between 97.63 – 114 % .It is evident from the content that the content was much higher in the inclusion complex formed with the molar ratio of 1:2. Moreover the % RSD for the inclusion complex formed in 1:2 molar ratio for both kneaded product and freeze dried product are more than 2 %. This might be the result of non-uniform distribution of drug in high amount of βCD. Uniform distribution of LER in βCD was observed in inclusion complexes formed in molar ratio of 1:1 and 1:1.5 as the results obtained are well within the prescribed limit.

Saturation Solubility Study

Results of saturation solubility study are shown in Table 5 and Fig. 3, which depicts increase in solubility of LER from all the inclusion complexes and physical mixture. The solubility of LER from physical mixture is more than the pure drug but the increase in solubility is not much higher suggesting that merely mixing the drug with cyclodextrin has not much effect on the solubility of LER. So the method of preparation of inclusion complex plays an important role in solubilising the drug. This may be because the proper method of preparation incorporates drug molecule into the cavity of cyclodextrin to hide the hydrophobic core of drug molecule.

However method of preparation also has significant effect on solubility as inclusion complex prepared by freeze drying method showed better solubility compared to kneaded complex. Also the amount of CD used for complex formation is an important factor to produce complex. Out of all molar ratio tried, molar ratio of 1:1.5 gave maximum solubility. LER solubility increased 5.48 fold when complexed with βCD and 7.7 fold when complexed with HPβCD [28].

***In vitro* dissolution study**

Dissolution study of inclusion complexes was studied in 1% SLS. The dissolution data obtained along with Dissolution Efficiency at 30 min (DE_{30}). Dissolution profiles of inclusion complexes and physical mixtures prepared with βCD and HPβCD are shown in Fig. 2 and 3. It is evident from the dissolution profiles that LER in the complex with both CD exhibited faster dissolution than LER pure powder. Increase in dissolution was observed in series Physical mixture < kneaded complex < freeze dried complex in both the Cyclodextrinused. It is clearly visible that the merely mixing the drug and cyclodextrin does not contribute much to the dissolution enhancement as the dissolution profile obtained with physical mixtures are similar to LER pure. LER in pure powder form showed only 50 % release over 60 min whereas the inclusion complex prepared by freeze drying in molar ratio of 1:1.5 showed 80% release in 20 minutes for both CD. The dissolution was much higher in 10 min for inclusion complexes formed by freeze drying method. Drug release pattern confirmed the results of saturation solubility as increase in the molar ration of 1:1.5 to 1:2 didn't show any significant increase in the dissolution behaviour of the drug. The effect of molar ratio is also evident from the dissolution profile as the dissolution was increased in series of 1:1<1:2<1:1.5. This suggests that the favourable interaction between Drug and Cyclodextrin takes place when the molar ratio is 1:1.5. Fig.5 shows the comparison of dissolution profile obtained with 1:1.5 molar ratios by freeze drying technique. Out of both the cyclodextrin tested, the inclusion complex formed with HPβCD shows promising result as the almost complete dissolution is achieved within 60minutes. Increase in dissolution of LER can be explained on the basis of the fact that presence of cyclodextrin in vicinity of the dissolving drug particle creates a "driving force" and rate of dissolution becomes proportional to this additional force resulting in the enhanced dissolution [29]. Dissolution of LER in inclusion complex is higher and enhanced as the poorly soluble LER exists in more hydrophilic environment when it is incorporated into thecyclodextrin cavity. The effect of HPβCD is more pronounced in dissolution enhancement owing to the fact that derivatized cyclodextrin inclusion complex are relatively more amorphous and has a higher solubility as water molecule can easily break up the amorphous molecule than the crystalline one [30] and in turndissolution.

The results of dissolution can be explained more prominently in terms of Dissolution Efficiency at 30 min (DE_{30}).Highest dissolution efficiency is obtained with the inclusion complex formed with HPβCD in a molar ration 1:1.5 by freeze drying technique. Hence, this formulation was compared with LER pure and marketed LER MKT in terms of dissolution profile, Mean Dissolution Time (MDT) and DE_{30}.

Final comparison of optimized inclusion complex was carried out with LER pure and LER marketed tablet using validated dissolution test procedure. The result of comparison is shown in Fig.6 Corresponding data for the same is depicted in Table 7 Table 8 shows comparison of mean dissolution time (MDT) and dissolution efficiency (DE_{30}) [31].

MDT obtained for optimized inclusion complex HPBCDF2 F_2 is lowest (7.42 min) indicating that rapid release of LER is obtained from inclusion complex as compared to LER marketed tablet (MDT-

13.64 min) and LER pure (MDT – 13.74 min). Also the DE_{30} of HPBCDF2 was found to be 76.06 % which is higher than LER pure (28.52 %) and LER marketed tablet (46.92 %). Dissolution Efficiency thus obtained indicates that all the three dissolution profiles are different and the same is supported by calculation of similarity factor (*f2*) in model independent method which is not within 50-100 as shown in Table 8.

Solid state characterization study

From solubility and *in vitro* dissolution result, the inclusion complex prepared by freeze drying technique in molar ratio of 1:1.5 was selected for further solid state characterization.

Fourier Transform Infrared Spectroscopy (FTIR)

Functional group and structure of organic compounds are well understood and predicted by FTIR study. Structure of prepared inclusion complex was studied in detail with the help of FTIR spectra. FTIR spectra of LER, βCD, Inclusion complex and physical mixture is depicted in Fig. 6B.6.LER (Fig. 7 (a)) shows characteristic peaks of N-H stretching vibration at 3202 cm^{-1}, C-O-CH_3 stretching vibration at 2772 cm^{-1}, C=O stretching vibration at 1681 cm^{-1} and C=C vibration at 1461 cm^{-1}. βCD (Fig. 3.7 (b)) shows , maximum absorption at 3227 cm^{-1} because of O-H bonds of primary hydroxyl groups,2920 cm^{-1} of C-H stretching , broad absorption band within 1400-1200 cm^{-1} corresponding to C-H vibrations. FTIR spectra of physical mixture of LER and βCD (Fig. 3.7 (c)) shows the peaks of LER at 3204 cm^{-1},3084 cm^{-1},1661 cm^{-1} and 1661 cm^{-1} which are undetected in the FTIR of inclusion complex. Absence of these peaks is explained by the complexation of drug into host moiety[8]. Moreover ,in the spectra of inclusion complex (Fig. 3.7 (d)), peaks of LER at 1520 cm^{-1}, 1485 cm^{-1},1344 $cm^{-1}$and 1232 cm^{-1} showed shifting to 1524 cm^{-1},1487 cm^{-1},1347 cm^{-1} and 1215 cm^{-1} . This shift supports the strong interactions taking place between LER and βCD and can be explained by the dissociation of the intermolecular hydrogen bonds associated with crystalline drug molecules [19]. These observations suggest possible entrapment of LER into cavities of βCD which is the indication of formation of inclusion complex of LER and βCD. This explains the presence of weak interaction between LER and βCD [32]. FTIR spectra of LER, HPβCD, Inclusion complex and physical mixture is depicted in Fig.3.7 As evident from the figure, characteristic peaks of LER at 3202 and 2772 cm^{-1} and few in fingerprinting region is visible in the spectrum of physical mixture (Fig 7 (c)). The FTIR spectrum of physical mixture is the characteristic spectra obtained by superimposing the spectra of LER and HPβCD. This observation suggests that merely mixing the drug with HPβCD is not enough to form inclusion complex. LER peak at 3184 cm^{-1} is completely disappeared in the FTIR spectra of inclusion complex formed by freeze drying technique (Fig 7 (d)). This observation suggests that strong interaction between LER and HPβCD takes place in the freeze dried inclusion complex. Disappearance of peak corresponding to N-H stretch indicates possible formation of hydrogen bond between N-H group of LER and HPβCD. This observation is supported by the findings of Prabhu et al.[33] and Medarevi et al [34].

Powdered X-ray Diffraction (PXRD)

XRD is most commonly used to confirm inclusion complex of βCD in powdered state. Fig. 8 depicts PXRD patterns of LER, Physical mixture and freeze dried inclusion complex with βCD and HPβCD. The diffraction pattern of inclusion complex differs from that of pure drug because of super position of peaks of both the components [32]. LER, βCD and HPβCD show multiple peaks at various 2θ in their PXRD pattern which suggests their crystalline structure. LER illustrated strong and sharp peaks at diffraction angle (2θ) of 7.0, 18.9, 23.1 and 24.9 outlining well defined crystal

structure.

In case of physical mixture of LER with βCD, PXRD pattern showed characteristic crystallinity peaks of drug and βCD both but with lesser intensity than that was obtained with pure compounds. Physical mixture PXRD pattern suggests that inclusion complex formation doesn't take place by simply triturating LER with βCD [35]. PXRD pattern of inclusion complex formed by freeze drying method showed presence of new peaks and loss of few peaks from LER and βCD which suggests change in crystalline structure of βCD. This observed change is attributed to the probable formation of inclusion complex [23].

When physical mixture of LER with HPβCD was analysed by PXRD, the pattern obtained showed significant decrease in crystallinity which might be attributed to the amorphous in nature of HPβCD. However in the physical mixture also few peaks at diffraction angle (2θ) 23.1 and 24.9 of LER is visible indicating the presence of crystalline structure. Whereas the PXRD pattern of inclusion complex showed complete absence of peak in the pattern which confirms the presence of only amorphous material in the inclusioncomplex. The results of PXRD studies indicated that conversion of crystalline to amorphous state of LER takes place when inclusion complex is formed with HPβCD by freeze drying technique.

^{1}H Nuclear Magnetic Spectrometry (NMR)

Proton NMR Spectroscopy is used to characterize inclusion complex based on the changes in chemical shifts of proton of both the drug and βCD. ^{1}HNMR spectra of LER, βCD, Physical mixture and inclusion complex is shown in Fig. 3.9. LER shows NMR signals corresponding to its proton over the δ values of 2-5 and 7-8. Similar signals along with basic signals of βCD are visible in NMR spectrum of physical mixture in Fig. 9 (c). However, chemical shifts of LER were decreased in intensity or went undetected in the NMR spectra of inclusion complex Fig. 9 (d).Also sharpness of signals have been reduced and widening is seen in the inclusion complex in comparison with LER and physical mixture spectra. Downfield change in the chemical shifts of proton of LER is clearly observed and reported in the Table 9 Thus overall behaviour of LER along with βCD in physical mixture and in inclusion complex suggests that a strong hydrophobic interaction is taking place between the cavity of βCD and a portion of LER which can be the result of incorporation of LER into the βCD. The formation of inclusion complex is also supported by the results obtained in FTIR and PXRD studies[22]. ^{1}H NMR studies result of inclusion complex HPBCDF2 is shown in Fig.10 along with NMR spectra of LER, HPβCD and physical mixtures of both. The data of chemical shift observed is indicated in Table 9The evident overlapping of peaks in region of 4-5 ppm and downfield shifting of NMR signal of host molecule collectively suggest strong hydrophobic interaction taking place between the LER and HPβCD [36].

Differential Scanning Calorimetry

From the result of FTIR, PXRD and ^{1}H NMR studies it was observed that out of the two inclusion complexes formed with βCD and HPβCD, the one prepared with HPβCD showed significant increase in dissolution and interaction with LER. Hence, the DSC studies were performed with the inclusion complex of HPβCD. DSC thermogram of LER, HPβCD, physical mixture and HPBCDF2 is depicted in Fig. 11 Details of thermodynamic parameters corresponding to the DSC thermograms are given in Table 10

DSC thermogram of LER shows sharp endothermic peal at 178.35 corresponding to its melting point and confirming its crystalline structure. HPβCD in its thermogram shows a shoulder at 81.21

º C. Physical mixture of HPβCD and LER shows overlapping of these two peaks but with reduced height, peak area and heat of fusion.

As seen from the DSC thermogram of inclusion complex, the second peak corresponding to LER which was seen in physical mixture is completely absent and also the shoulder at 80.20 º C is appearing with further decrease in peak height, peak area and heat of fusion than those observed with physical mixture. All above observation collectively supports the strong interaction between LER and HPβCD converting crystalline LER to amorphous complex with enhanced solubility and better dissolution.

Stability and Photostability Study

Based on the results obtained for solubility and *in vitro* dissolution, inclusion complex of LER prepared with HPβCD by freeze drying technique in the ratio of 1:1.5 was subjected to stability study. The % drug content and % cumulative drug release obtained after storage at 40ºC/75% RH is shown in Table 11 and profile for the same is depicted in Fig. 12. Drug content of solid dispersion was found to be in range of 97.10 to 98.4 after storage. Drug release after storage is also unaltered after storage. Similarity factor for all the duration studied for stability was in the range 50-100 indicating that the drug release pattern after stability is similar to the initial release. The results obtained for stability study indicates that the solid dispersion produced is stable for six months.

Optimized inclusion complex of Lercanidipine hydrochloride with hydroxyl propyl beta cyclodextrin showed loss of crystallinity of Lercanidipine hydrochloride in XRD pattern (Fig 12 (a)) [36]. The XRD pattern obtained for the optimized inclusion complex after storage of 6 months (Fig 12(b)) demonstrated same XRD pattern as that of freshly prepared inclusion complex indicating that the crystalline behaviour of Lercanidipine hydrochloride is not reappeared. The detailed study of XRD patterns confirms that the change in crystalline behaviour is not changed even after storage for 6 months. Photostability studies depicted major effect of UV light on solid state of LER and its formulation while solution state was more sensitive to sunlight. Results of photostability confirmed the reported photostability issue of LER and hence all the experiments were conducted in the amber colored glass apparatus.

CONCLUSION

The study carried out in this work suggests that solubility and dissolution of LER can be enhanced by incorporating it into HPβCD using freeze drying method.- From the result obtained it can be concluded that out of physical mixing , kneading and freeze drying technique, freeze drying method is most successful in producing inclusion complex with faster and higher dissolution. Evaluation of inclusion complexes formed with various molar ratio suggested that, successful inclusion complex can be formed with the molar ratio of 1:1.5. Significant increase in the DE_{30} and MDT was obtained with the optimized inclusion complex. Result of FTIR, PXRD, ^{1}H NMR and DSC confirms that strong interaction takes place between LER and HPβCD.

TABLE 1. Composition and coding of Inclusion complexes of LER with βCD and HPβCD

Formulation code	CD used	Method of preparation	Molar Ratio
BCDK1	β Cyclodextrin (βCD)	Kneading	1:1
BCDK2			1:1.5
BCDK3			1:2
BCDF1		Freeze drying	1:1
BCDF2			1:1.5
BCDF3			1:2
BCDPM1		Physical Mixture	1:1
BCDPM2			1:1.5
BCDPM3			1:2
HPBCDK1	Hydroxy Propyl β Cyclodextrin (HPβCD)	Kneading	1:1
HPBCDK2			1:1.5
HPBCDK3			1:2
HPBCDF1		Freeze drying	1:1
HPBCDF2			1:1.5
HPBCDF3			1:2
HPBCDPM1		Physical Mixture	1:1
HPBCDPM2			1:1.5
HPBCDPM3			1:2

TABLE 2 Stability Constants (Ks) and slope for LER solid dispersions-

Type of Cyclodextrin	Slope	Stability Constant (M^{-1})
β-Cyclodextrin	0.0049	428.18
Hydroxy Propyl β-Cyclodextrin	0.0091	798.571

TABLE 3 Phase solubility and ΔG°_{tr} of LER at different concentrations of βCD and HPβCD

Concentration of βCD (mM)	Concentration of LER (mM)*	ΔG°_{tr} (JK^{-1}	Concentration of HPβCD (mM)	Concentration of LER (mM)*	ΔG°_{tr} (JK^{-1}

		mol^{-1})			**mol^{-1})**
4.409	0.0280±0.00030	-2285.28	3.4	0.0435±0.00051	-3414.10
8.818	0.0472±0.00045	-3624.65	6.84	0.0830±0.00057	-5079.24
13.227	0.0796±0.00062	-4971.61	10.27	0.1084±0.00089	-5768.80
17.63	0.1007±0.00087	-5578.61	13.69	0.1362±0.00114	-6356.46
22.04	0.1186±0.00123	-6000.12	17.12	0.1706±0.00136	-6936.93
26.45	0.1397±0.00178	-6422.75	20.54	0.1952±0.00125	-7283.19
30.86	0.1581±0.00145	-6740.87	23.97	0.2370±0.00240	-7783.57
R^2	0.994		0.997		
Type of curve	A_L		A_L		

*Data expressed at Mean ± SD (n=3)

TABLE 4 Content of Inclusion Complexes of LER

Formulation	**Content (%w/w) (Mean ±SD)***
BCDF1	102.87±1.59
BCDF2	101.52±1.55
BCDF3	114.64±2.06
BCDK1	98.60±1.69
BCDK2	101.20±0.89
BCDK3	111.35±2.24
BCDPM1	101.36±1.23
BCDPM2	99.95±0.98
BCDPM3	97.69±0.85
HPBCDF1	98.39±1.12
HPBCDF2	101.52±1.08
HPBCDF3	100.89±1.65
HPBCDK1	102.36±1.87
HPBCDK2	103.87±0.97
HPBCDK3	99.87±0.67

HPBCDPM1	97.63±0.78
HPBCDPM2	100.23±1.36
HPBCDPM3	98.69±1.47

***Data expressed at Mean ± SD (n=3)**

TABLE 5 Saturation solubility of LER and inclusion complexes

S. N.	Inclusion Complexes	Solubility in distilled water (mg/mL)*
1.	LER	0.0510±0.0023
2.	BCDF1	0.2045±0.0022
3.	BCDF2	0.2792±0.0037
4.	BCDF3	0.2238±0.0033
5.	BCDK1	0.1013±0.0012
6.	BCDK2	0.1438±0.0022
7.	BCDK3	0.1215±0.0019
8.	BCDPM1	0.0712±0.0014
9.	BCDPM2	0.0845±0.0016
10.	BCDPM3	0.0756±0.0012
11.	HPBCDF1	0.3029±0.0044
12.	HPBCDF2	0.3925±0.0070
13.	HPBCDF3	0.3264±0.0053
14.	HPBCDK1	0.2133±0.0021
15.	HPBCDK2	0.2405±0.0045
16.	HPBCDK3	0.2208±0.0034
17.	HPBCDM1	0.1526±0.0028
18.	HPBCDM2	0.1948±0.0031
19.	HPBCDM3	0.1786±0.0025

***Data expressed at Mean ± SD (n=3)**

TABLE 6 *In vitro* dissolution data for LER pure and inclusion complex formed with βCD andHPβCD

Formulati	10*	20*	30*	40*	50*	6	DE_{30}

on						0 *	
LER	33.19±0.041	37.07±0.183	46.93±0.065	47.07±0.041	47.43±0.012	50.00±0.401	31.24
BCDF1	17.69±0.118	55.51±0.196	57.88±0.237	66.99±0.408	68.02±0.126	71.81±0.721	34.05
BCDF2	53.22±0.538	80.05±0.885	81.97±0.816	82.30±1.271	84.86±0.706	87.47±0.845	58.08
BCDF3	19.85±0.210	57.01±0.124	60.15±0.211	62.21±0.719	69.24±0.641	70.50±0.532	35.65
BCDK1	16.65±0.24	51.19±0.711	51.83±0.816	55.43±0.222	55.63±0.288	56.50±0.286	31.25
BCDK2	30.55±0.499	52.67±0.573	54.79±0.774	60.28±0.731	68.32±0.491	70.87±0.816	36.87
BCDK3	17.82±0.240	47.35±0.244	49.16±0.276	50.95±0.241	51.33±0.274	51.8830.283	29.92
BCDPM1	34.91±0.860	42.64±0.410	47.21±0.232	47.60±0.372	49.4520.291	51.76±0.225	33.72
BCDPM2	36.67±0.286	46.04±0.312	47.59±0.349	51.47±0.424	54.54±0.657	55.32±0.737	35.50
BCDPM3	37.482±0.926	47.81±0.032	49.83±0.040	53.2200.109	55.30±0.188	60.21±0.392	36.73
HPBCDF1	58.26±0.360	69.60±0.449	70.30±0.481	71.86±0.728	74.66±0.228	75.42±0.617	54.34
HPBCDF2	75.89±0.408	87.56±0.816	93.65±0.489	99.01±0.449	99.41±0.816	99.51±0.849	76.05
HPBCDF3	57.63±0.244	63.13±0.817	67.15±0.739	70.64±0.854	72.56±0.870	73.26±0.597	51.44
HPBCDK1	47.53±0.598	51.38±0.482	51.97±0.113	52.75±0.354	53.41±0.563	60.23±0.563	41.63
HPBCDK2	54.66±0.513	61.78±0.439	67.24±0.780	70.23±0.808	71.22±0.813	73.26±1.23	50.02
HPBCDK3	41.71±0.400	45.38±0.430	47.08±0.454	49.54±0.816	51.32±0.64	53.26±0.98	36.87
HPBCDPM1	25.45±0.297	41.64±0.555	47.20±0.638	48.67±0.792	50.56±0.716	51.14±1.002	30.23
HPBCDPM2	31.17±0.363	48.94±0.545	56.3±0.75131	62.23±1.069	64.70±0.655	67.56±0.871	36.08
HPBCDPM3	23.63±0.409	34.50±0.646	38.02±0.676	43.86±0.583	45.10±0.420	48.69±0.959	25.71

***Data expressed at Mean ± SD(n=3)**

TABLE 7 *In vitro* release data of HPBCDF2, LER MKT and LER pure in 0.1 N HCl at 100 rpm

Time (min)	HPBCDF2*	LER MKT*	LER Pure*
0	0	0	0
5	74.36±1.21	28.36±0.48	22.36±0.42
15	81.75±1.23	44.00±0.71	31.10±0.37
30	93.65±1.85	86.00±0.57	47.35±0.83
45	99.32±1.74	92.51±1.08	55.39±0.81
60	99.51±1.65	94.01±1.23	58.27±0.91
MDT (min)	7.42	13.64	13.74
DE (%)	76.06	46.92	28.52

***Data expressed at Mean ± SD (n=3)**

TABLE 8 Comparison of Dissolution profiles

Comparison	Similarity Factor	Dissolution Profile
LER Pure and HPBCDF2	19.39	Dissimilar
Marketed tablet formulation and HPBCDF2	30.30	Dissimilar

TABLE 9 Chemical shifts (ppm) for LER protons and LER-βCD inclusion complex

δ LER	δ BCDF2	ΔδBCDF2	δ HPBCDF2	Δδ HPBCDF2
7.61	8.02	0.41	7.96	0.35
7.59	7.97	0.38	7.91	0.32
7.54	7.6	0.06	7.61	0.02
7.52	7.58	0.06	7.59	0.07
7.5	7.56	0.06	7.48	-0.02

TABLE 10 Thermal behaviour of DSC Thermograms of LER pure, HPβCD, Physical Mixture and optimized Inclusion Complex (HPBCDF2)

System	Peak point °C	Peak height (mW)	Peak area (mJ)	Heat of fusion (J/g)
LER	178.35	4.1373	189.493	73.56
HPβCD	81.21	1.18	247.35	91.34
Physical Mixture peak 1	80.03	1.03	228.92	83.15
Physical Mixture peak 2	192.51	0.88	29.538	10.72

HPBCDF2	80.20	0.74	167.86	60.20

TABLE 11 Drug content and i*n vitro* dissolution stability data of optimized solid dispersion after storage at 40ºC/75% RH

Time (min)	Cumulative Drug Release*			
	Initial	60 days	120 days	180 days
5	74.36±1.21	73.86±1.51	72.47±0.98	71.38±1.87
15	81.75±1.23	80.25±1.74	79.54±1.65	78.45±2.69
30	93.65±1.85	92.15±1.32	91.65±1.87	90.10±3.64
45	99.32±1.74	97.48±1.46	96.89±1.64	95.64±4.21
60	99.51±1.65	98.12±2.10	97.48±2.30	96.78±4.44
Similarity factor between initial release and release after stability	-	89.30 Similar	83.09 Similar	75.12 Similar
Drug Content (%w/w)	99.34±0.43	98.75±0.67	97.18±1.4	96.84±0.9

***Data expressed at Mean ± SD (n=3)FIGURE 1 Phase solubility diagram of LER with different concentration of βCD and HP Bcd**

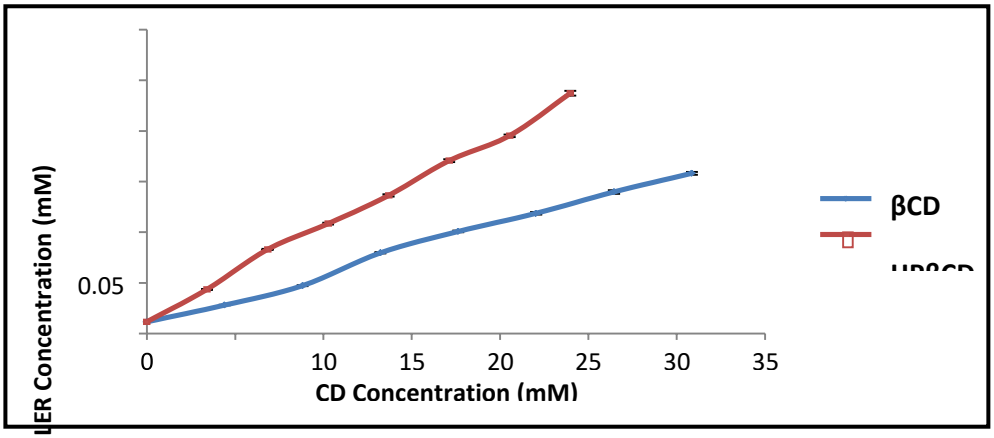


FIGURE 2 *In vitro* dissolution profile of inclusion complexes formed with βCD

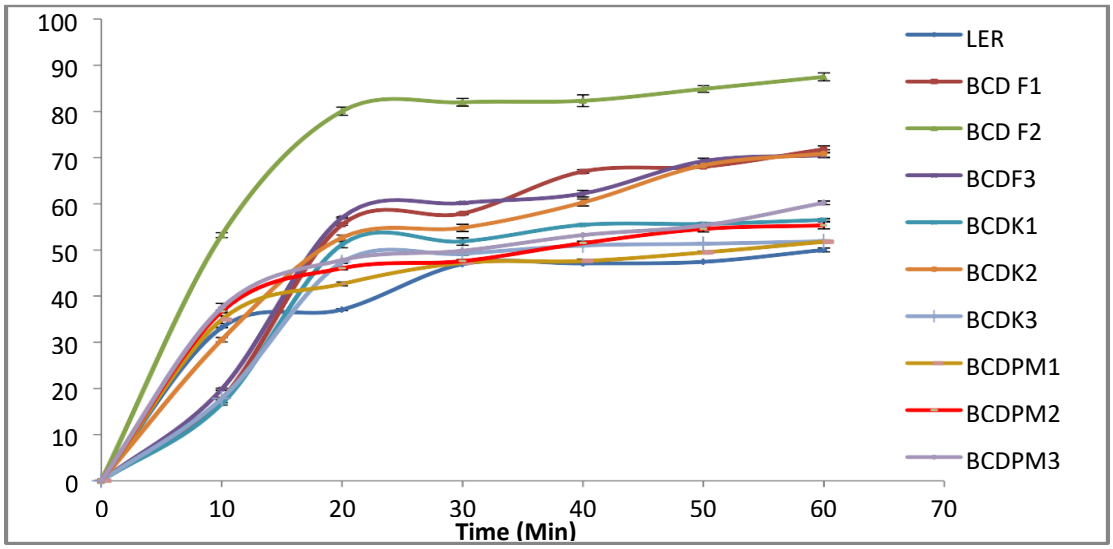


FIGURE 3 *In vitro* dissolution profile of inclusion complexes formed with HPβCD

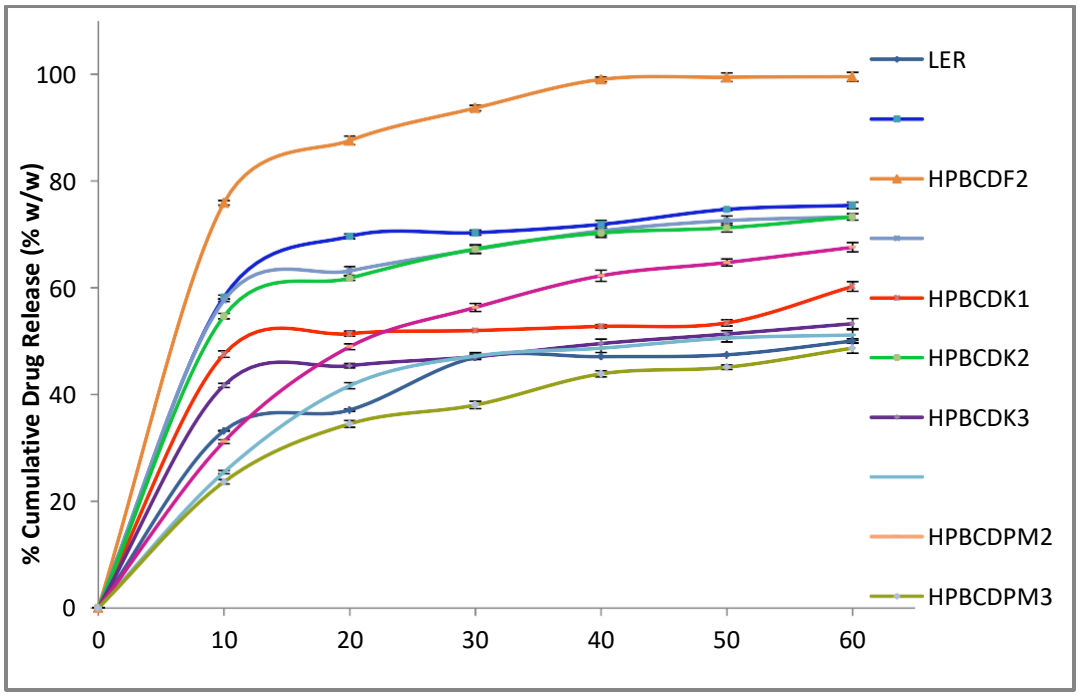


FIGURE 4 *In vitro* dissolution profile of inclusion complexes formed in a molar ratio of 1:1.5 and LER pure

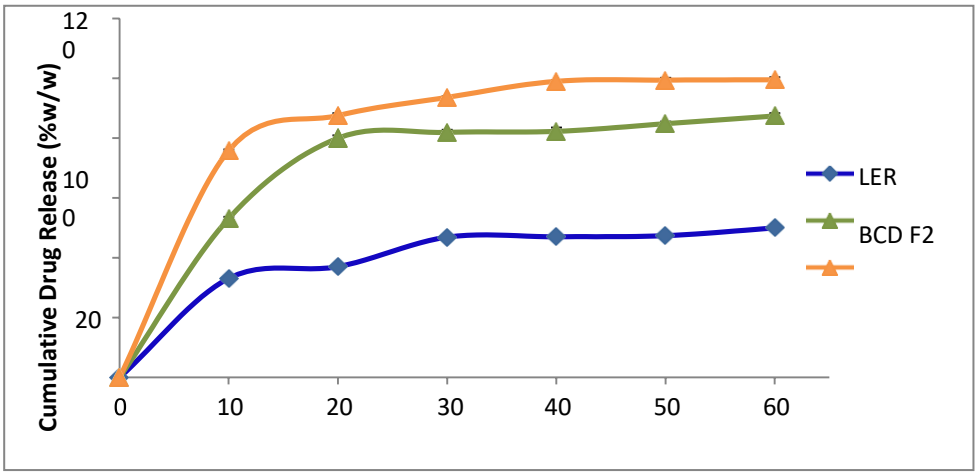


FIGURE 5 Dissolution profile of HPBCDF2, LER pure and LER MKT in 0.1 N HCl at 100 rpm

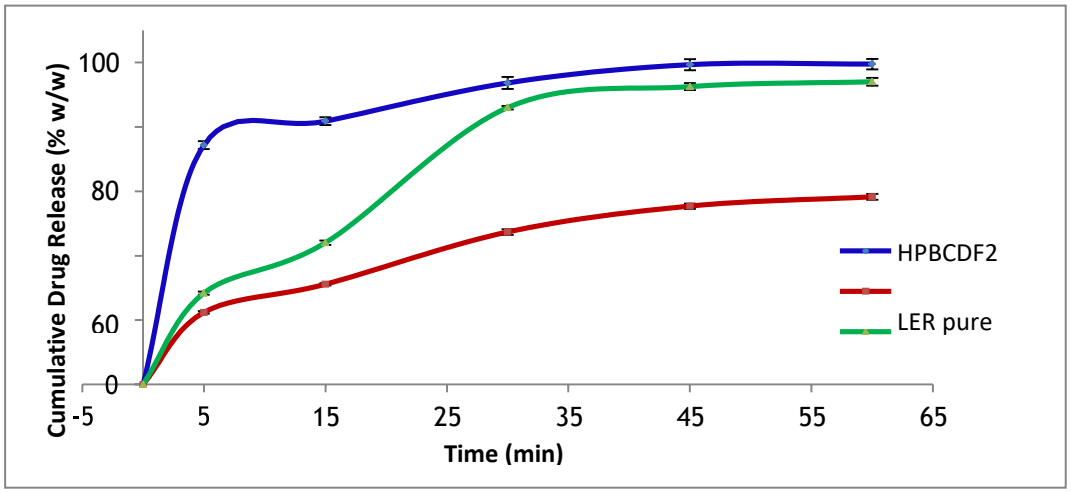


FIGURE 6 FTIR spectrum of (a) LER (b) βCD (c) Physical mixture of LER : βCD (1:1.5) (d) Freeze dried inclusion complex of LER: βCD (1:1.5)

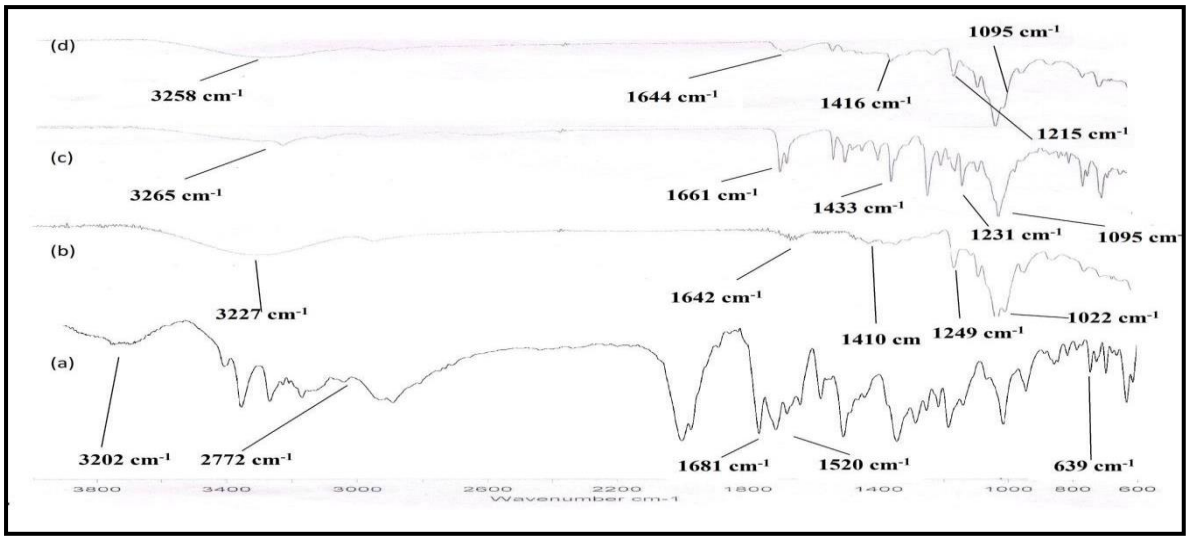


FIGURE 7 FTIR Spectra of (a) LER (b) HPβCD (c) Physical Mixture LER: HPβCD (1:1.5) (d) HPBCDF2

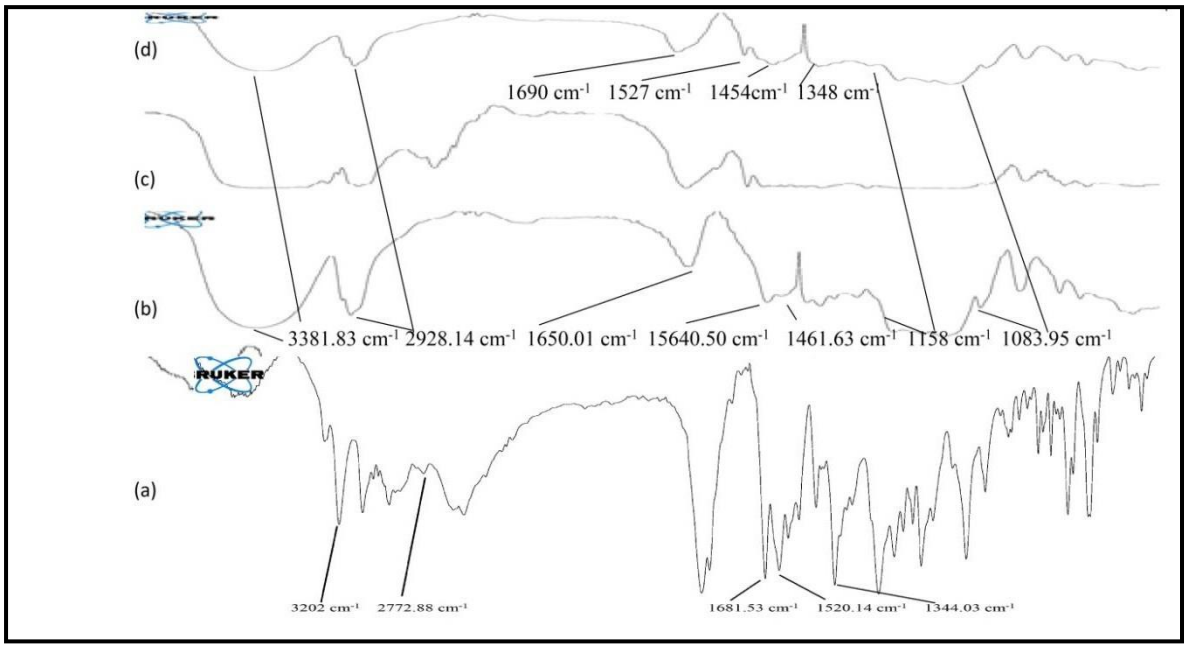


FIGURE 8 XRD Pattern of (a) LER (b) Physical Mixture LER : βCD (1:1.5) (c) BCDF2(d) Physical Mixture LER : HPβCD (1:1.5) (e) HPBCDF2

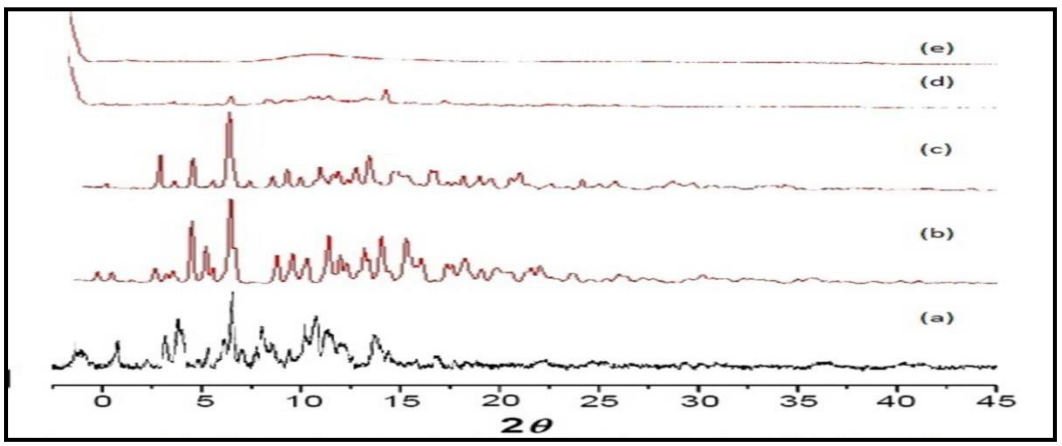


FIGURE 9 ^{1}H NMR spectra of (a) LER (b) βCD (c) Physical mixture (d) Inclusion complexBCDF2

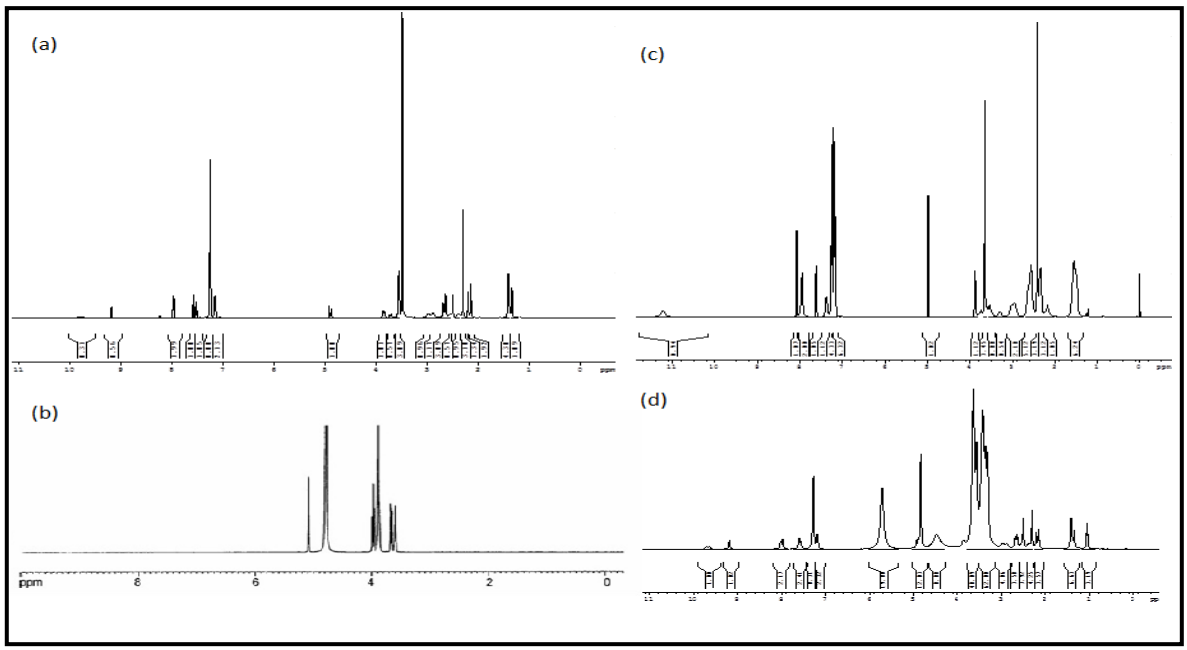


FIGURE 10 ^{1}H NMR spectra of (a) LER (b) HPβCD (c) Physical mixture (d) Inclusion complex HPBCDF2

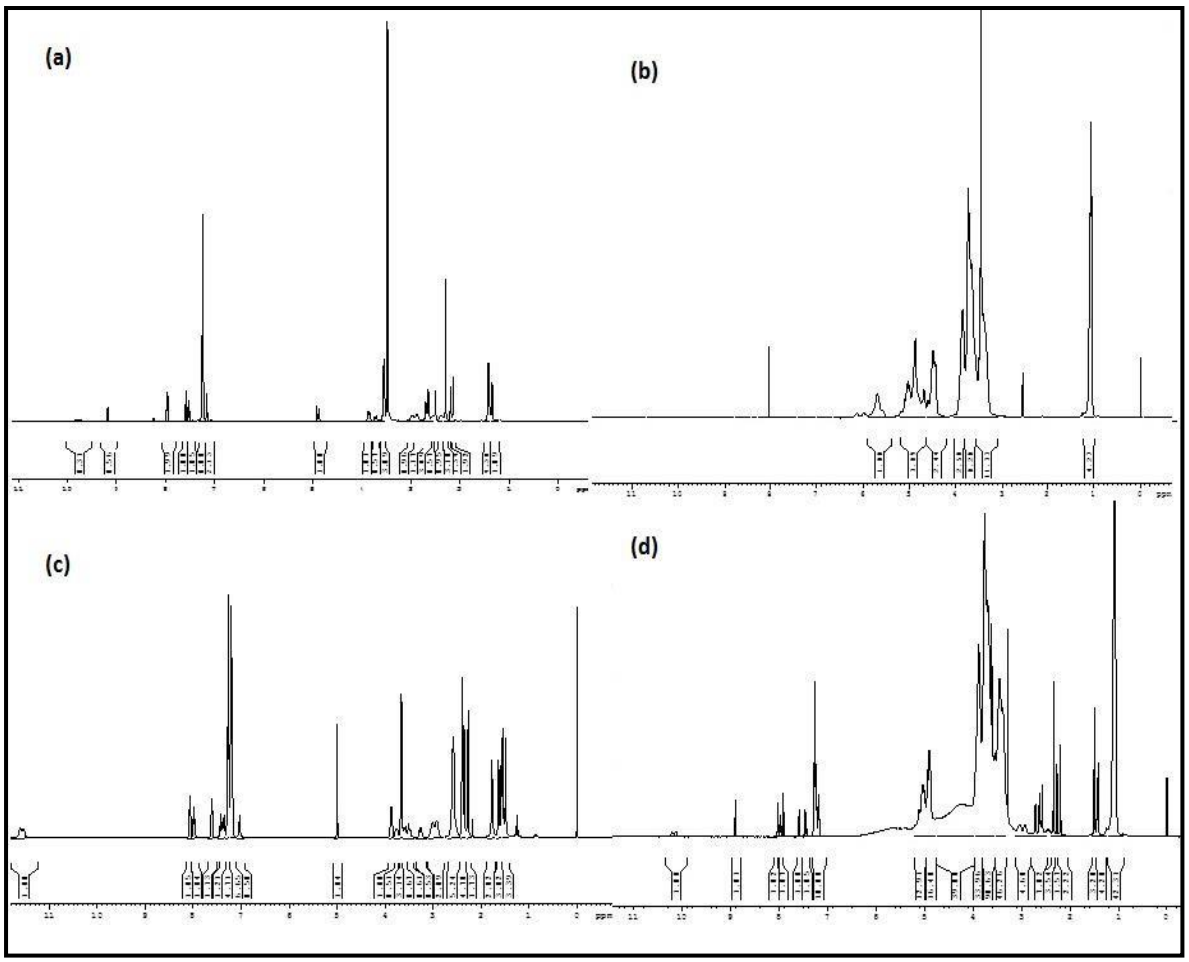

(a)
(b)
(c)
(d)

FIGURE 11 DSC thermogram of (a) LER (b) HPβCD (c) Physical mixture with HPβCD (1:1.5) (d) HPBCDF2

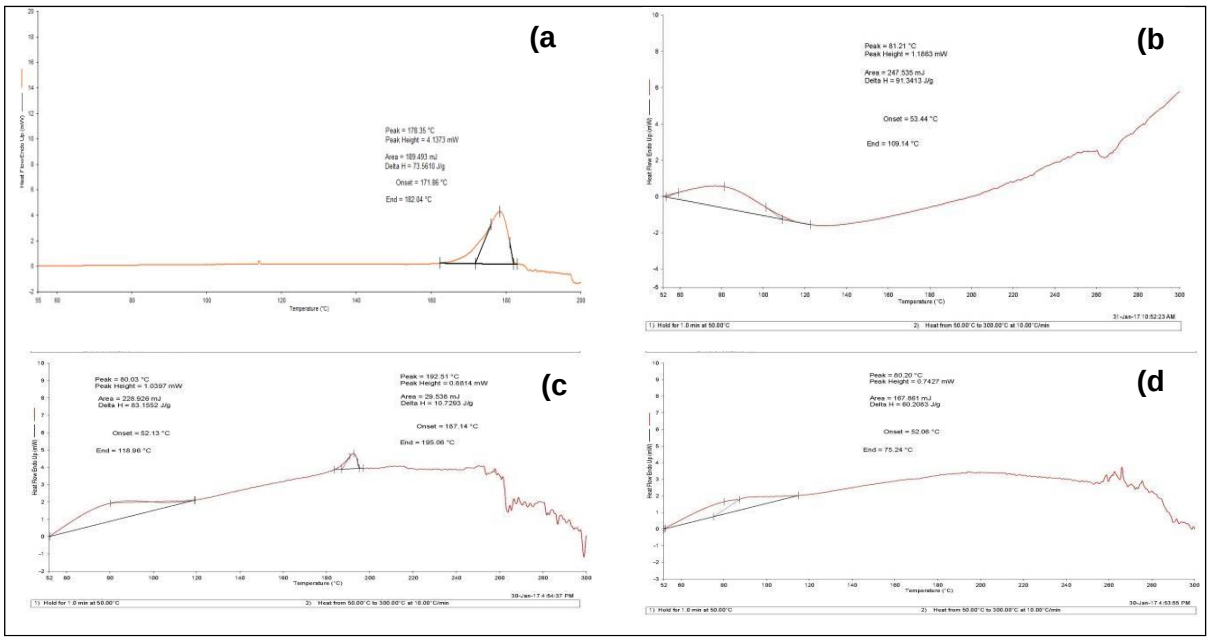


FIGURE 12 Dissolution profile of optimized Inclusion complex after stability study

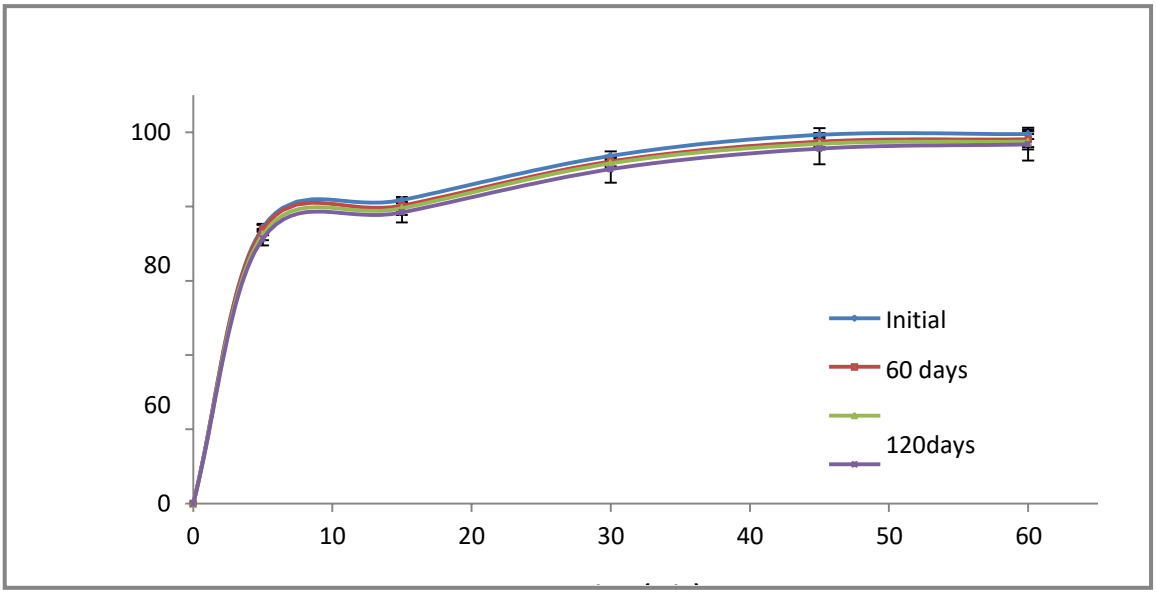
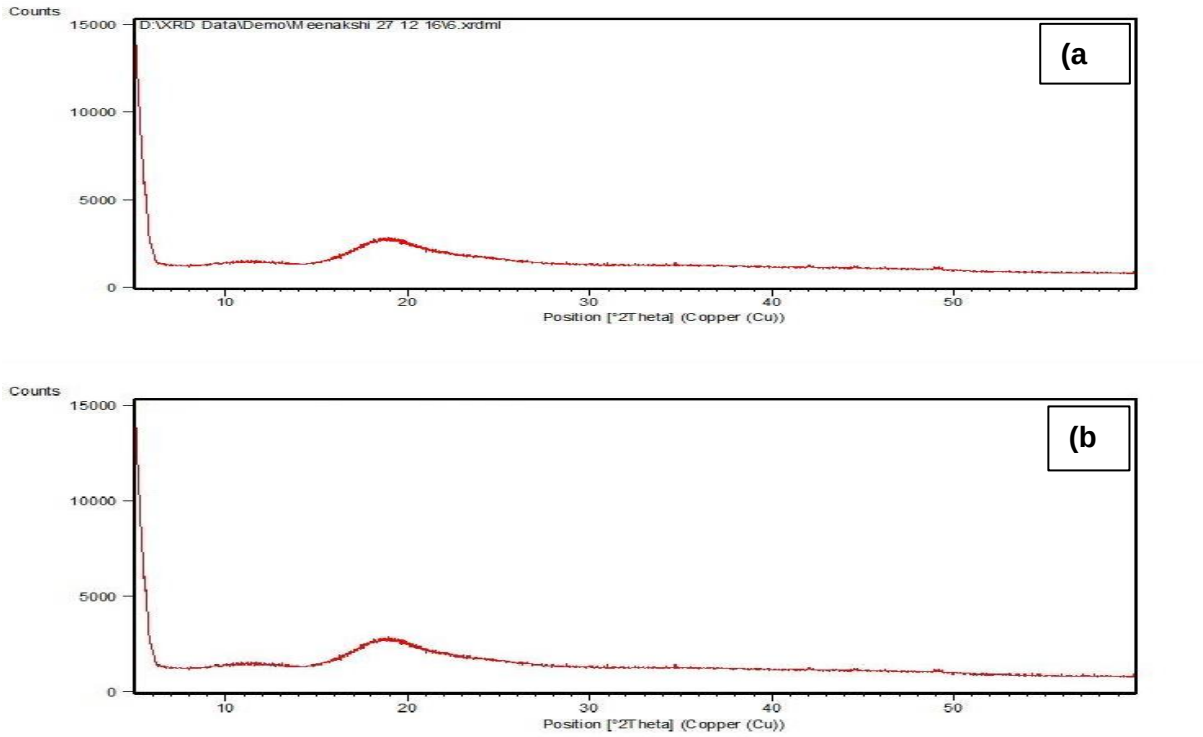


FIGURE 12 Powdered X Ray diffraction patterns of Optimized inclusion complex of LER and HPBCD (a) at zero time (b) after storage of 6 months



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