VERIZONA PUBLISHER
Publishing Your Ideas

www.verizonaonlinepublishing.com
Vol: 1, Issue: 1

Journal of Pharma and Pharmaceutical Sciences

Enhanced Dissolution Efficiency of Zaleplon Solid Dispersions via Modified β-Cyclodextrin Molecular Inclusion Complexes

Carmen Popescu[1*], Prashanth Manda[2], Abhishek Juluri[2], Karthik Yadav Janga[3], Manasa Cidda[3] and S. Narasimha Murthy[2,4]

[1]Roquette America Inc., Geneva, Illinois 60134 USA

[2]Department of Pharmaceutics and Drug Delivery, University of Mississippi 38677

[3]University College of Pharmaceutical Sciences, Kakatiya University, India 506009

[4]Institute for Drug Delivery and Biomedical Research, Bangalore, India

***Corresponding author:** Carmen Popescu, Senior Project Coordinator, Roquette America Inc., Geneva IL, USA, E-mail: carmen.popescu@roquetteamerica.com

Article Type: Research, **Submission Date**: 30 March 2015, **Accepted Date:** 8 May 2015, **Published Date:** 29 May 2015.

Citation: Carmen Popescu, Prashanth Manda, Abhishek Juluri, Karthik Yadav Janga, Manasa Cidda, et al. (2015) Enhanced Dissolution Efficiency of Zaleplon Solid Dispersions via Modified β-Cyclodextrin Molecular Inclusion Complexes. J.Pharm Pharm Scien 1(1): 12-21. doi: https://doi.org/10.24218/vjpps.2015.04.

Abstract

The focus of the current study is to confirm the potential of chemically modified cyclodextrins (methyl-β-cyclodextrin (MβCD/Kleptose® Crysmeb), hydroxypropyl-β-cyclodextrin (HP-β-CD/Kleptose® HPB) and sulfobutylether-β-cyclodextrin (SBE-β-CD/Captisol®)) to improve the dissolution rate of zaleplon in oral delivery. Native and modified cyclodextrins were screened via phase solubility studies in order to select the most efficient cyclodextrin in formation of stable inclusion complexes. A_L-type phase solubilization resulted from phase solubility diagrams of all cyclodextrin indicated a linear proportional relation between the solubility of zaleplon versus different CD concentrations. Complexation with crysmeb and HP-β-CD resulted in greater complexation constant values and higher percentage complexation efficiency values. Therefore these two cyclodextrins were selected for formulation development. Inclusion complexes of zaleplon:cyclodextrin solid dispersions were prepared using lyophilization and spray-drying techniques. DSC and XRD performance delineates the transformation of crystalline form of zaleplon to amorphous state. ^{1}HNMR studies confirm the presence of drug in the hydrophobic cavity of cyclodextrins and reveal the amorphous nature of the formulations. The stability studies of inclusion complexes for 60 days at 25°C/60 % RH and 40°C/75%RH resulted in low or no variations in the percentage of complexation efficiency suggesting good stability of molecular complexes. In conclusion, *in vitro* dissolution studies displayed an overall two-fold improvement in the dissolution rate compared to pure drug attributed to Crysmeb and HP-β-CD as suitable complexing agents in enhancing the solubility of zaleplon.

Keywords: Zaleplon, Cyclodextrins, Complexation efficiency, DSC, *in vitro* dissolution, ^{1}HNMR

Introduction

Limited solubility of therapeutic entities is a great challenge for many formulators in the pharmaceutical industry. Most of the new chemical entities and existing drug molecules demonstrate poor solubility indicating less dissolution efficiency and in turn low bioavailability [1]. To enhance the aqueous solubility of lipophilic moieties formulation scientists have investigated myriad of traditional and novel approaches like salt formation, solid dispersions, eutectic mixtures, micronization, lipid drug delivery systems, colloidal delivery systems, hot melt extrusion, etc [2-5]. Only few of the aforementioned strategies were scalable-up resulting in a formulation with desired dissolution behavior. A well-recognized strategy in practice for more than a century is formation of inclusion complexes by molecular encapsulation in the hydrophobic cavity of cyclodextrins (CD). Numerous products in the market developed by pharmaceutical industries are using this technology [6].

CD are (α-1,4)-linked oligosaccharides of α-D-glucopyranose with fairly spatial hydrophobic internal cavity responsible for non-covalent inclusion of suitable drug and hydrophilic exterior, imparting interactions with water triggering APIs so as to improve the solubility [7]. Among α, β and γ CD the most commonly considered into formulation technology are β-CD, as it results in dosage forms with improved dissolution efficiency [6]. Hampered aqueous solubility of native β-CD had given advantage for chemical modification by substitution with alkyl groups like methyl, hydroxypropyl and sulfobutyl ether resulting in enormous amplification in solubility with good safety profile [8]. Modified β-CD have gained wider applications in delivering the drugs (BCS class II and IV) via different routes of administration due to their increased solubilizing effect resulting in stable inclusion complexes and improved permeation across biological membranes [9,10].

Zaleplon, a BCS class II drug, has low bioavailability (<30%) with limited aqueous solubility (~0.1605 mg/mL) [11]. It is a pyrazolopyrimidine hypnotic drug prescribed for treatment and management of Insomnia. There is proven evidence of its therapeutic efficiency as a benzodiazepine due to GABA receptor interaction [12]. It is a potent anticonvulsant in pentylenetetrazole and electroshock induced convulsions. Encapsulation of zaleplon

in pro-liposomes and self-emulsifying systems improved dissolution and consequently enhanced bioavailability [13,14].

The purpose of the present research is to increase zaleplon's solubility by formulating it as solid dispersions of molecular inclusion complexes with methyl-β-CD (Crysmeb) and hydroxypropyl-β-CD (HP-β-CD). Phase solubility studies are performed to determine the solubilizing propensity of zaleplon by βCD, Crysmeb, HP-β-CD and SBE-β-CD via inclusion complexes followed by their stability evaluation at 25˚C/60% RH & 40˚C/70% RH. Complexation constant ($K_{1:1}$) and complexation efficiency (CE) were calculated based on phase solubility curves. Lyophilization (Lyo) and spray drying (SD) techniques were investigated and optimized to obtain solid dispersions of zaleplon in CD solutions. In order to understand zaleplon's transformation from crystalline form to an amorphous state and the interaction of drug with CD's, solid state characterization studies were performed. *In vitro* dissolution studies were carried out to evaluate the ability of the optimal CD and solid dispersion preparation method to improve zaleplon's solubility by creating stable inclusion complexes.

Materials and Methods

Materials

Zaleplon was a generous gift sample from Maven Life Sciences Pvt. Ltd and Symed Labs, Hyderabad, India. Native β-cyclodextrin (βCD), Hydroxypropyl-β-cyclodextrin (HP-β-CD/Kleptose® HPB), Methyl-β-cyclodextrin (Kleptose ® Crysmeb) were provided by Roquette America, Inc. (Geneva,Illinois,USA). Sulfobutylether-β-cyclodextrin (SBE-β-CD/Captisol®) was provided from Cydex Inc. (Lenexa, Kansa, USA). All other chemicals and solvents used were of analytical grade and HPLC grade respectively. Deionized (D.I) water used throughout the experiments.

Methods

HPLC Analysis: High performance liquid chromatography (HPLC) system (Waters, 1525) associated with an auto sampler (Waters, 717 plus) and equipped with a variable wavelength dual λ absorbance detector (Waters, 2487) used for quantification of zaleplon according to the established method [14]. 20µL of sample was isocratically eluted in mobile phase of aqueous solution and acetonitrile (45:55 v/v) at a flow rate of 1.0 mL/min through a Phenomenex, Luna, C18 (2) 100 R analytical column (4.6 mm x 150 mm, 5.0 µm) at 32˚C. The column effluent was monitored at 232 nm.

Phase Solubility Studies: Increasing concentrations of HP-β-CD, Crysmeb, SBE-β-CD solutions (0, 5, 10, 20, 30, 40 & 50 mM/L) and β-CD solutions (0, 2,4,8,12 & 16 mM/L) respectively, were utilized in performing phase solubility studies as reported by Higuchi & coworkers [15]. In brief, to 15 mL of CD solution excess amount of drug was added and then subjected to shaking at room temperature for a period of 7 days. Supernatant of these solutions were filtered through a 0.45µm filter membrane **(Millipore, Billerica MA)** prior to estimation of drug by HPLC. The obtained CD solutions with drug were exposed to 25˚C/60% RH & 40˚C/70% RH and evaluated for complexes stability for a period of 60 days. In addition of these solutions were lyophilized. Experiments were executed in triplicates.

The complexation constant ($K_{1:1}$) and complex efficiency (CE) were calculated from linear proportionality of phase solubility diagrams (Figure 1), according to equations (1) and (2) respectively.

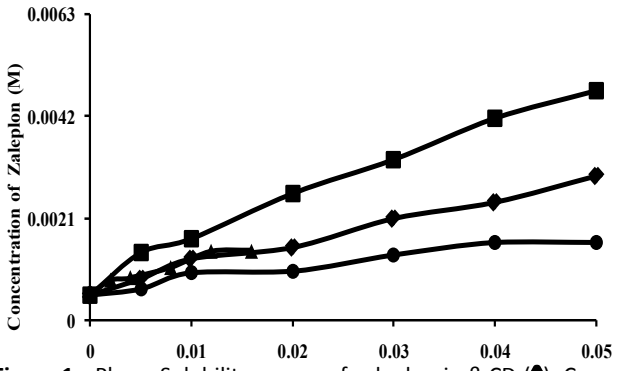

Figure 1: Phase Solubility curves of zaleplon in β-CD (◆), Crysmeb (■), HP-β-CD (▲) and SBE- β-CD (●) at RT(25°C) and Deionized water (pH=6.5)

$$K_{1:1} = \frac{\text{Slope}}{S_o(1-\text{Slope})} \quad (1)$$

$$CE = S_o * K_{1:1} = \frac{\text{Slope}}{(1-\text{Slope})} \quad (2)$$

Where S_0 is the intrinsic solubility of drug in water and Slope is slope of the solubility curve

Preparation of CD solid dispersions

Solid dispersion by Lyophilization (Lyo): Based on phase solubility graphs an A_L-type means that the complexation ratio CD: zaleplon is on 1:1 molar basis. Briefly, 0.45g zaleplon was dissolved separately in acetone and methanol and then added drop by drop to the aqueous solutions of Crysmeb (1.78g) and HP-β-CD (2.1g) respectively. The resulting clear solutions were frozen at -160˚C under liquid nitrogen and subsequently dried at -45˚C in a freeze dryer (Labconco, FreeZone 4.5, Kansas City, MO) under vacuum (pressure of 185 $x10^{-3}$ Mbar) in order to obtain a dry powder of CD solid dispersion formulations of zaleplon-crysmeb-lyophilised (Zal-Crys-Lyo) and zaleplon-HP-β-CD-lyophilised (Zal-HP-β-CD-Lyo).

Solid dispersion by Spray Drying (SD): Spray drying technique is a very popular technique to prepare solid dispersions. The solutions of inclusion complexes (processed as above in freeze-drying technique) were spray dried in a spray dryer (Buchi 190 mini sprayer, Switzerland) operated at a feed rate of 30% with inlet and outlet temperatures of 78˚C and 55˚C respectively. The airflow compressed at a rate of 600 L/h with 90% of aspirator. The resultant spray dried solid dispersions of zaleplon-crysmeb-spray dried (Zal-Crys-SD) and Zaleplon-HP-β-CD-spray dried (Zal-HP-β-CD-SD) formulations were evaluated for their solubilization efficiency.

Physical Mixture (PM): Zaleplon-crysmeb-physical mixture (Zal-Crys-PM) and Zaleplon-HP-β-CD-physical mixture (Zal- HP-β-CD-PM) were prepared for comparative study with

respect to CD solid dispersions produced by lyophilization and spray drying. Equimolar quantities of drug and CD's were mixed in Turbula System Schats (Willy a. Bacheofen AG, Basel, Switzerland) to get drug: CD complex molar concentration 1mol:1mol as per phase solubility evaluation.

Drug content in solid dispersion powder: To analyze the drug content in the resultant formulations (solid dispersions and physical mixtures), appropriate quantities were weighed (in triplicate) and then dissolved in mobile phase. The resulting solutions were vortexed and filtered through 0.45µm filter membrane (Millipore, USA). The clear solutions were injected into HPLC for drug quantification.

Solid State Characterization

Differential scanning calorimetry (DSC): CD solid dispersions, physical mixtures, Crysmeb, HP-β-CD and pure drug were assessed by DSC analysis on Perkin-Elmer Diamond (Shelton, CT) Differential scanning calorimeter calibrated with indium for melting point and heat of fusion in order to evaluate the crystalline nature of drug. Samples were accurately loaded into aluminum pans, sealed and heated at a temperature ranging from 30°C to 300°C with a constant nitrogen gas flow of 30 ml/min at a heating rate of 20°C /min. The peak area in melting endotherms of zaleplon from formulations and pure API gives the heat of fusion (ΔH) value, which further is used to describe the degree of crystallinity (D_{crys}) of drug measured by means of following equation (3) [16].

$$Dcrys = f\frac{\Delta H_F}{\Delta H_D} \times 100 \qquad (3)$$

Where ΔH_F, ΔH_D and 'f' of equation (3) represents heat of fusion of drug in formulations (CD solid dispersions), heat of fusion of pure drug and fraction of drug contained in mixture respectively.

Powder X-ray Diffractometry (PXRD): X-ray diffractometer (Bruker D8-Focus XRD, Netherlands) reveals the PXRD patterns of pure drug, Crysmeb, HP-β-CD and CD solid dispersions. The samples were operated with a Cu anode tube over the interval of 5-70°/2θ at 40 kV voltage and 30 mA current. 10°/min scanning speed was constant throughout the process.

Proton nuclear magnetic resonance spectroscopy (^{1}HNMR): To assess the interaction between the drug and CD, ^{1}HNMR spectra of Zaleplon, Crysmeb, HP-β-CD and formulations were recorded with the aid of proton nuclear magnetic resonance spectroscopic studies on Brucker Ultraspec 400 MHz/100MHz spectrometer (Brucker Biospin Corporation, The Woodlands, TX, USA) operated with methanol as a solvent at a frequency of 300MHz.

In vitro dissolution study: To illustrate the effectiveness of cyclodextrins in enhancing the dissolution behavior of drug, CD solid dispersions (**Zal-Crys-Lyo, Zal-Crys-SD, Zal-HP-β-CD-Lyo and Zal-HP-β-CD-SD),** physical mixtures (**Zal-Crys-PM and Zal-HP-β-CD-PM**)) and pure drug were assessed by *in vitro* dissolution tests. The tests were run in triplicate using USP type II (paddle) apparatus (Hanson SR-8 plus, Hanson Research Corporation, Chatsworth, CA) in 500mL phosphate buffer saline (pH 6.75) dissolution medium maintained at 37±0.5°C temperature with paddle speed set at 75 rpm right through the study. Relevant quantities of formulations (CD solid dispersions and physical mixtures) and pure active equivalent to 5mg dose were placed into dissolution media. Aliquots of 5mL were withdrawn at preset time intervals followed by refilling with fresh media to retain fixed volume. Before the evaluation of drug by HPLC, the collected samples were filtered through 0.45µm filter membrane (Millipore, USA).

Dissolution parameters: The percentage cumulative amount of drug released in 5 and 30 minutes (Q_5 and Q_{30} respectively) were derived from the *in vitro* dissolution release profile. Dissolution efficiency (DE) was depicted from the area under dissolution curve illustrated by means of trapezoidal rule and represented as a percentage in the area of rectangle representing 100% dissolution in the same time. It is calculated by using the following equation (4): [17].

$$DE = \frac{\int_o^t y\,dt}{y_{100}t} X\ 100\% \qquad (4)$$

Dissolution rate considered in first 15 minutes of dissolution was ensued as initial dissolution rate (IDR). IDR is calculated according to following equation (5):

$$IDR = \frac{\%\ \text{dissolved}}{\text{min}} \qquad (5)$$

One more dissolution parameter is Mean dissolution time (MDT) which exemplifies the probable time taken for dissolving a molecule from solid dosage form. Hence, MDT is defined as the average time for the drug to dissolve under *in vitro* dissolution conditions. Equation (6) used to calculate MDT [18]:

$$MDT = \frac{\sum_{j=1}^{n} t_j \Delta M_j}{\sum_{j=1}^{n} \Delta M_j} \qquad (6)$$

Where *j* is the *in vitro* dissolution sample number, n defines number of sample time points, t_j is the midpoint of the j^{th} time period easily calculated with [t + (t−1)]/2 and ΔM_j is the additional amount of drug dissolved between t_j and t−1.

The sum of drug coming into bulk of dissolution media per unit time under *in vitro* dissolution conditions is defined as the mean dissolution rate (MDR). This is calculated by following equation (7) [18]:

$$MDR = \frac{\sum_{j=1}^{n} \Delta M_j / \Delta t}{n} \qquad (7)$$

Where ΔM_j is the additional amount of drug dissolved between t_j and t−1, n is the number of *in vitro* dissolution sample times, Δt the midpoint time between t_j and t−1 which can be easily calculated with (t + t−1)/2.

The ratio of dissolution efficiencies of individual formulations (CD solid dispersions/ physical mixtures) to pure drug will demonstrate the relative dissolution rate (RDR) (equation 8) [19].

$$RDR = \frac{DE_{Formulation}}{DE_{Pure\ drug}} \quad (8)$$

Stability Studies: The aliquots of phase solubility studies were placed in stability chambers at 25°C/60% RH and 40°C/75%RH for period of 60days. Samples were withdrawn at 0, 30 and 60 days from the study, processed and estimated for the content of drug by HPLC to predict the % CE with respect to 0 day CE.

Table 1: Phase Solubility and Complexation Efficiency Studies of zaleplon in aqueous solutions of β-cyclodextrin (β-CD), Methyl β-cylodextrin (Crysmeb), Hydroxypropyl β-Cylodextrin (HP-β-CD) and Sulfobutyl ether β-Cylclodextrin (SBE-β-CD) at 25°C.

CD	Conc. of CD (mM)	Solubility of Zal. (S) (mg/mL)	Solubility of Zal (M)	S/So	$K_{1:1}$	CE
β-Cyclodextrin	0	0.1605 ±0.0014	0.00053	1.00	88.78	0.046
	2	0.2536 ±0.0011	0.00083	1.58		
	4	0.2671±0.0002	0.00087	1.66		
	8	0.3329±0.0018	0.001	2.07		
	12	0.4322±0.0022	0.0014	2.69		
	16	0.4386±0.0021	0.0014	2.73		
Crysmeb (Methyl β-Cyclodextrin	0	0.1605 ±0.0014	0.00053	1.00	157.55	0.082
	5	0.4236±0.0012	0.0013	2.64		
	10	0.5102±0.0018	0.0016	3.18		
	20	0.7999±0.0021	0.0026	4.98		
	30	1.1779±0.0043	0.0039	7.34		
	40	1.2665±0.0057	0.0041	7.89		
	50	1.4467±0.0062	0.004	9.01		
HP-β-CD (HydroxyPropyl β-Cyclodextrin)	0	0.1605±0.0014	0.00053	1.00	93.38	0.049
	5	0.2653±0.0011	0.00087	1.65		
	10	0.3869±0.0016	0.00126	2.41		
	20	0.4578±0.0012	0.0015	2.85		
	30	0.6371±0.0011	0.002	3.97		
	40	0.7408±0.0025	0.0024	4.62		
	50	0.9104±0.0029	0.0029	5.67		
SBEβ-CD (Sulfo Butyl Ether β-Cyclodextrin	0	0.1605±0.0014	0.00052	1.00	53.38	0.028
	5	0.1989±0.0013	0.00065	1.24		
	10	0.3005±0.0016	0.00098	1.87		
	20	0.3125±0.0022	0.001	1.95		
	30	0.4091±0.0017	0.0013	2.55		
	40	0.488±0.0025	0.0015	3.04		
	50	0.4874±0.0029	0.0015	3.04		

Where **CD** (Cyclodextrins); m**M** (Millimolar); S_o (Solubility of zaleplon in water); **S** (Solubility of zaleplon in aqueous solutions of various CD's); S/S_o (Solubility increase ratio); $K_{1:1}$ (Complexation constant); **CE** (Complexation Efficiency).

Results and Discussion

Phase Solubility Studies

The intrinsic solubility of drug (S_o), its aqueous solubility in CD solutions along with $K_{1:1}$ and CE are reported in Table 1. The phase solubility studies results suggest the positive impact of CD's in augmenting (S/S_0) the aqueous solubility of zaleplon (0.1605±0.0014 mg/mL) (Table 1). The phase solubility diagram (PSD) of zaleplon in CD solutions displays an A_L-type graph with regression values closer to 1 in all cases pointing the linear proportional increment in the solubility of drug with increasing concentration of CD's (Figure 1) [15]. The slope values less than unity in PSD attributed a first order complexation phenomenon indicating the equimolar (1mol:1mol) complexation between drug and CD [20] (Figure 1). A solubility of 9 fold, 5 fold and 3 fold improvement with Crysmeb, HP-β-CD and SBE-β-CD respectively over a 2 fold rise in aqueous solubility of drug with native cyclodextrin (β-CD) is due to chemical modification of native CD [21] (Table 1). The $K_{1:1}$ value is an experimental factor to characterize the performance variations of CD in improving the solubility of drug [6]. The chemical modification of native β-CD with smaller groups like methyl (Crysmeb) and hydroxyl propyl (HP-β-CD) had resulted with higher $K_{1:1}$ values of 157.55 and 93.38 respectively which perhaps owing to the larger cavity space with hydrophobic nature [9] (Table 1). β-CD substituted with bulkier group like sulfobutyl ether (SBE-β-CD) have shown the least value of complexation constant (53.38) ensuring prospect of steric interaction and reduced hydrophobic factors between host and guest molecule obviating the inclusion of drug [6]. To overcome the sensitivity errors expected from PSD in determining the solubility, CE was estimated suitably for choosing appropriate CD for formulation development. The CE values of β-CD, Crysmeb, HP-β-CD and SBE-β-CD were ~0.046, 0.082, 0.049 and 0.026 respectively suggests that the stronger inclusion complexes are formed by Crysmeb and HP-β-CD.

Based on the results obtained from phase solubility studies, Crysmeb and HP-β-CD were selected as suitable CD's for formulation development due to their superior solubilizing capacity. Moreover, earlier reports suggests that the chemically modified beta cyclodextrins (Crysmeb and HP-β-CD) have greater applicability in development of oral solid dosage forms due to higher efficiency of complexation and lower toxicity over native β-cyclodextrin [22].

Drug content analysis

The ranking of zaleplon solubilization in various CD solutions are as follows: Crysmeb>HP-β-CD> β-CD>SBE- β-CD stating the higher solubilizing effect of Crysmeb as reported earlier [23]. Freeze-drying and spray drying techniques were investigated in order to formulate solid dispersions of drug with Crysmeb and HP-β-CD. The drug content in all formulations is depicted in Table 2. The values shown for solid dispersions were in correlation with the theoretical values whereas the deviation found in physical mixtures is perhaps a reflection of the drug loss during sieving.

Solid-State characterization

Although the increased solubility of drug via CD was because of complex formation, it is uncertain to claim the presence of drug in the hydrophobic core of CD's without any external interaction. DSC, ^{1}HNMR and XRD studies were performed to verify the formation of inclusion complexes.

DSC analysis is a method to exemplify interactions between drug and CD's in solid state [24]. DSC summary of zaleplon, crysmeb, HP-β-CD, physical mixtures and CD solid dispersions were illustrated in Figure 2. A sharp melting peak at 188.22°C in thermogram of drug with 100% degree of crystallization (D_{crys}) value attributes to crystalline nature of zaleplon (Table 3). Anhydrous form of Crysmeb was evident from thermogram with the melting peak at 127.30°C whereas a broad peak at 122.66°C in the thermogram of HP-β-CD clarifies amorphous state assigned due to water loss [6]. Physical mixtures with both CD's have shown a melting peak of drug displaced to lower temperatures (Zal-Crys-PM (182.18°C) and Zal- HP-β-CD-PM (184.30°C)) with slighter D_{crys} values (Zal-Crys-PM (4.57) and Zal-HP-β-CD-PM (4.84)) addressing chemical interactions between drug and CD's during the complexation process [9] (Table 3).

Lyophilized solid dispersions has displayed a peak within the melting transition of drug but the lower degree of crystallization values of 3.34 and 4.40 for Zal-Crys-Lyo and Zal-HP-β-CD-Lyo respectively explains the transformation of drug to amorphous state [21] (Table 3). DSC profile of spray dried CD solid dispersions (Zal-Crys-SD and Zal-HP-β-CD-SD) exhibited no peak at drug melting point signifying the drug enclosed in cavity of CD to achieve inclusion complex and consequently amorphous

Table 2: Drug content analysis in formulation and physical mixtures

Formulations	Avg Wt (mg)	Drug content (mg)	Amount of Formulation ~10 mg of drug (mg)
Zal-Crys-PM	2.39±0.32	0.33±0.007	70.62±2.15
Zal-Crys-Lyo	2.40±0.05	0.43±0.003	54.75±1.36
Zal-Crys-SD	2.45±0.19	0.36±0.007	67.29±1.41
Zal-HP-β-CD-PM	2.27±0.20	0.26±0.003	84.79±4.67
Zal-HP-β-CD-Lyo	2.17±0.15	0.59±0.006	36.70±2.05
Zal-HP-β-CD-SD	2.49±0.18	0.36±0.002	69.07±2.85

Where: **Zal** (Zaleplon); **Zal-Crys-PM (**Zaleplon Crysmeb solid dispersion by physical mixture**); Zal-Crys-Lyo (**Zaleplon Crysmeb solid dispersion by lyophilization); **Zal-Crys-SD (**Zaleplon Crysmeb solid dispersion by Spray drying**); Zal-HP-β-CD-PM (**Zaleplon Hydroxypropyl β-Cylodextrin solid dispersion by physical mixture**); Zal-HP-β-CD-Lyo (**Zaleplon Hydroxypropyl β-Cylodextrin solid dispersion by lyophilization**); Zal-HP-β-CD-SD (**Zaleplon Hydroxypropyl β-Cylodextrin solid dispersion by Spray drying**).**

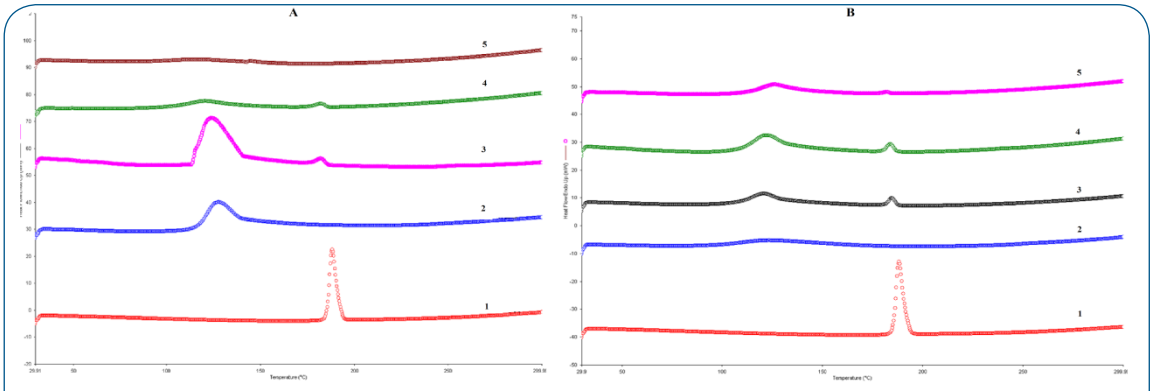

Figure 2: DSC thermograms of 1) Zaleplon, 2) Cyclodextrins (A. Crysmeb and B. HP-β-CD), 3) Physical Mixtures (A. Zaleplon-Crysmeb and B. Zaleplon- HP-β-CD), 4) Lyophilized solid dispersions (A. Zaleplon-Crysmeb and B. Zaleplon-HP-β-CD) and 5) Spray dried solid dispersions (A. Zaleplon-Crysmeb and B. Zaleplon- HP-β-CD).

Table 3: Degree of crystallinity of drug in formulations and physical mixtures obtained from DSC studies.

Formulations	Fraction of drug (f)	ΔHeat of fusion of drug (J/g) (ΔH_{SD})	$\%D_{crys}$
Zal	1	106.34 (ΔH_{dr})	100
Zal-Crys-PM	0.5	9.73	4.57
Zal-Crys-Lyo	0.5	7.11	3.34
Zal-Crys-SD	0.5	0.0	0.0
Zal-HP-β-CD -PM	0.5	10.29	4.84
Zal-HP-β-CD -Lyo	0.5	9.36	4.40
Zal-HP-β-CD -SD	0.5	1.63	0.77

Where: **Zal** (Zaleplon); **Zal-Crys-PM (**Zaleplon Crysmeb solid dispersion by physical mixture)**; Zal-Crys-Lyo (**Zaleplon Crysmeb solid dispersion by lyophilization); **Zal-Crys-SD (**Zaleplon Crysmeb solid dispersion by Spray drying)**; Zal-HP-β-CD-PM (**Zaleplon Hydroxypropyl β-Cylodextrin solid dispersion by physical mixture)**; Zal-HP-β-CD-Lyo (**Zaleplon Hydroxypropyl β-Cylodextrin solid dispersion by lyophilization)**; Zal-HP-β-CD-SD (**Zaleplon Hydroxypropyl β-Cylodextrin solid dispersion by Spray drying).

Where ΔH_{dr} (heat of fusion of pure drug); ΔH_{SD} (heat of fusion of drug in solid dispersions/physical mixtures) and $\%D_{crys}$ (% drug crystallinity).

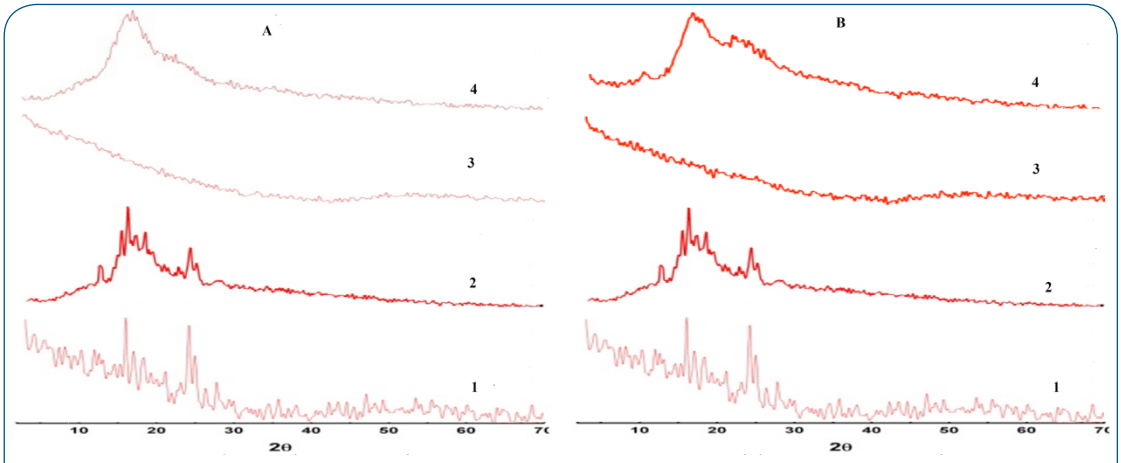

Figure 3: XRD-Diffractograms of 1) Zaleplon, 2) Physical mixtures (A. Zaleplon- HP-β-CD-PM and B. Zaleplon-Crysmeb-PM), 3) Lyophilized solid dispersions (A. Zaleplon- HP-β-CD- and B. Zaleplon-Crysmeb) and 4) Spray dried solid dispersions (A. Zaleplon- HP-β-CD- and B. Zaleplon-Crysmeb).

state [20] (Figure 2).

Figure 3 reveals diffractometric profiles of drug, Crysmeb, HP-β-CD and CD solid dispersions. The multitude of typical sharp diffraction peaks of zaleplon at 2θ of 14.06, 17.42, 19.52, 23.56 and 25.37 with good intensity designates the crystalline state of pure drug. Diffractograms of the entire formulations (except physical mixtures) demonstrated the nonexistence of sharp peaks disclosing the occurrence of amorphous state of drug upon complexation [20]. The common characteristics of diffraction peaks from physical mixture and pure drug reveal the presence of drug in crystalline state.

The quality and geometry of CD inclusion complexes can be extremely mediated by means of high sensitive NMR (^{1}HNMR) spectroscopy [25]. ^{1}HNMR spectra of pure drug exhibited a single proton (H-1) of methyl group on amide ring of phenyl ring at 1.933. H-2 and H-3 protons (as triplets) at 3.82 and 1.15 respectively represent the methylene and methyl functionalities of N-Substituted aromatic ring (Figure 4). Three doublet protons of aromatic ring at 7.49, 7.75 and 7.58 assigned as H-4, H-5 and H-6 respectively. The indication of two pyrimidine protons (8.16 and 8.84) and a single proton on pyrazole ring (8.65) were expressed as H-7, H-8 and H-9 respectively (Figure 4). The proton at H-3 and H-5 positions of Crysmeb (at 3.46 and 3.87) and HP-β-CD (at 3.57 and 3.82) delineates the internal cavity. There was a significant change in the position of protons at H-2, H-4 and H-5 of zaleplon in physical mixtures. Diffused proton peaks at H-1 and H-2 positions of drug in lyophilized and spray-dried formulations connote loss of crystalline nature of drug. The broad diffused peaks with unimportant shift in the position of H-3 and H-5 of Crysmeb and HP-β-CD in lyo and SD solid dispersions typifies the inclusion of guest molecule in the cavity of CD [26]. Our findings were in correlation with the earlier reports that have illustrated the penetration of lipophilic moieties into the cavity of CD through the brim on spacious side attributing to the chemical shifts in ^{1}HNMR spectra of H-3 and H-5 [27]. In contrary, only shift in either of protons (H-3/H-5) of Crysmeb and HP-β-CD protons denotes the inefficiency of complex development [28].

In vitro Dissolution studies

Dissolution is an excellent tool to spot the exact purpose of CD in enhancing the zaleplon' s solubility . The cumulative drug release profiles of zaleplon, physical mixtures (**Zal-Crys-PM and Zal-HP-β-CD-PM**) and CD solid dispersion formulations **(Zal-Crys-Lyo, Zal-Crys-SD, Zal-HP-β-CD-Lyo and Zal-**

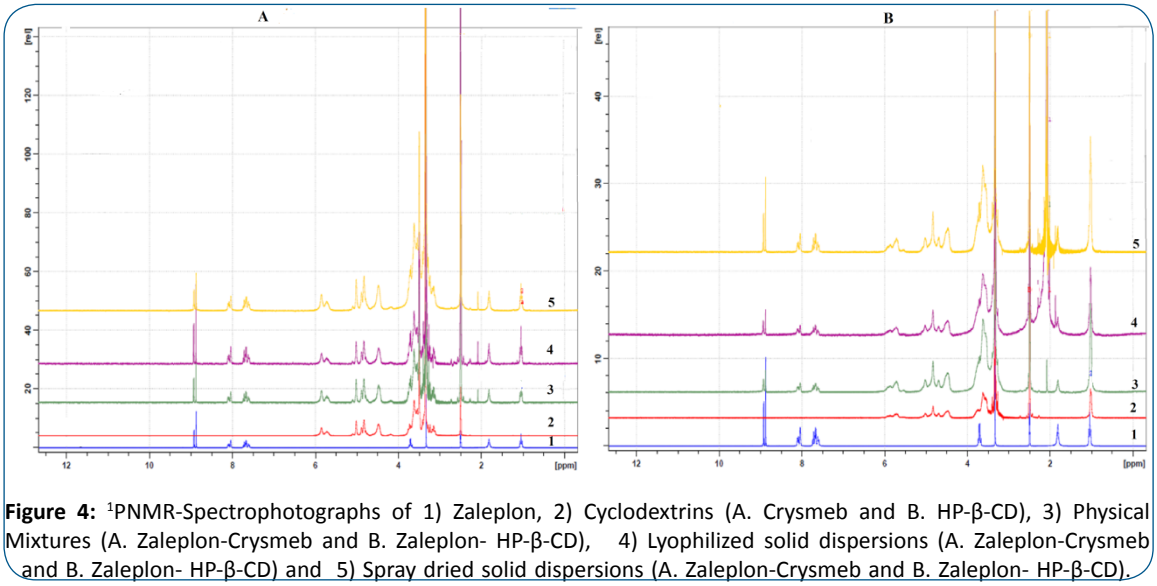

Figure 4: ^{1}PNMR-Spectrophotographs of 1) Zaleplon, 2) Cyclodextrins (A. Crysmeb and B. HP-β-CD), 3) Physical Mixtures (A. Zaleplon-Crysmeb and B. Zaleplon- HP-β-CD), 4) Lyophilized solid dispersions (A. Zaleplon-Crysmeb and B. Zaleplon- HP-β-CD) and 5) Spray dried solid dispersions (A. Zaleplon-Crysmeb and B. Zaleplon- HP-β-CD).

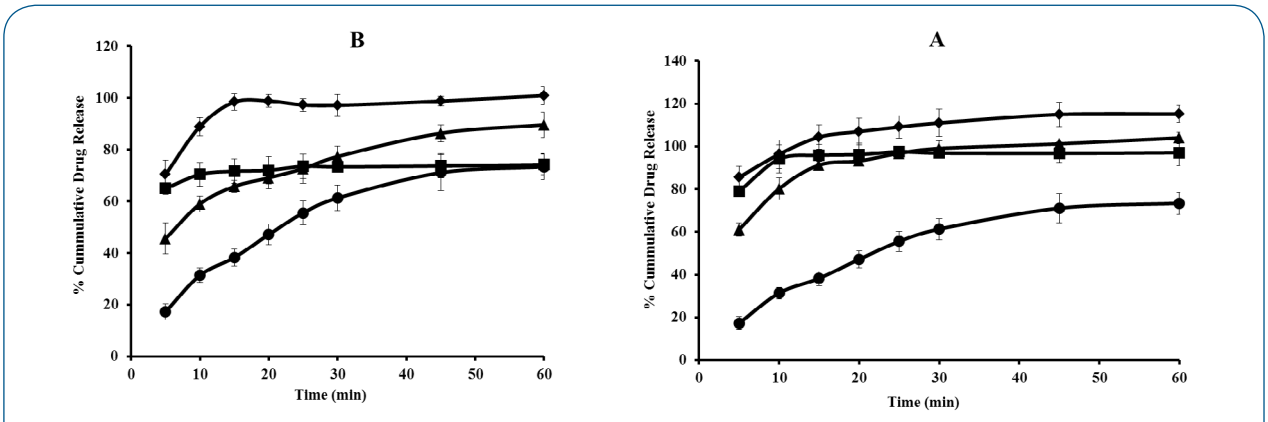

Figure 5: *In vitro* dissolution profile of A. Zaleplon Crysmeb solid dispersions (Zaleplon (●), Zaleplon Crysmeb PM (▲), Zalpelon Crysmeb Lyo (■), and Zaleplon Crysmeb SD (◆)); and B. Zaleplon HP-β-CD solid dispersions (Zaleplon (●), Zaleplon HP-β-CD PM (▲), Zalpelon HP-β-CD Lyo (■), and Zaleplon HP-β-CD SD (◆)).

Table 4: *In vitro* dissolution parameters of zaleplon from Lyophilized and Spraydried Solid dispersion with Crysmeb and HP-β-CD

Formulations				Dissolution parameters			
	Q_5	Q_{30}	DE	MDT	MDR	IDR	RDR
Zal	17.33±3.01	61.31±4.96	26.48±3.36	16.71±1.02	1.79±0.21	3.46±0.12	-
Zal-Crys-PM	61.10±3.21	99.02±3.63	45.00±2.95	8.07±1.95	4.72±0.36	12.22±0.20	1.81
Zal-Crys-Lyo	79.05±1.59	96.86±2.04	45.51±1.26	3.75±0.53	5.63±0.16	15.81±1.02	1.83
Zal-Crys-SD	85.57±5.24	101.97±6.41	51.74±4.91	6.09±0.84	6.12±1.53	17.11±1.23	2.08
Zal-HP-β-CD -PM	45.61±5.97	77.34±4.10	35.85±3.35	12.01±1.10	3.56±0.34	9.12±0.54	1.24
Zal-HP-β-CD -Lyo	65.00±2.25	73.35±2.09	34.66±2.41	3.97±1.01	4.48±0.11	13.00±0.98	1.79
Zal-HP-β-CD -SD	70.41±5.41	97.18±4.21	45.67±4.01	5.71±1.03	5.23±0.91	40.08±2.51	1.84

Where: **Zal** (Zaleplon); **Zal-Crys-PM (**Zaleplon Crysmeb solid dispersion by physical mixture)**; Zal-Crys-Lyo (**Zaleplon Crysmeb solid dispersion by lyophilization); **Zal-Crys-SD (**Zaleplon Crysmeb solid dispersion by Spray drying)**; Zal-HP-β-CD-PM (**Zaleplon Hydroxypropyl β-Cylodextrin solid dispersion by physical mixture)**; Zal-HP-β-CD-Lyo (**Zaleplon Hydroxypropyl β-Cylodextrin solid dispersion by lyophilization)**; Zal-HP-β-CD-SD (**Zaleplon Hydroxypropyl β-Cylodextrin solid dispersion by Spray drying).

Q_5 (% Cumulative drug released in 5 min); **Q_{30}** (% Cumulative drug released in 30 min); **DE** (Dissolution efficiency)**; MDT** (Mean dissolution time); **MDR** (Mean dissolution rate); **IDR (**Initial dissolution rate) **and RDR** (Relative dissolution rate).

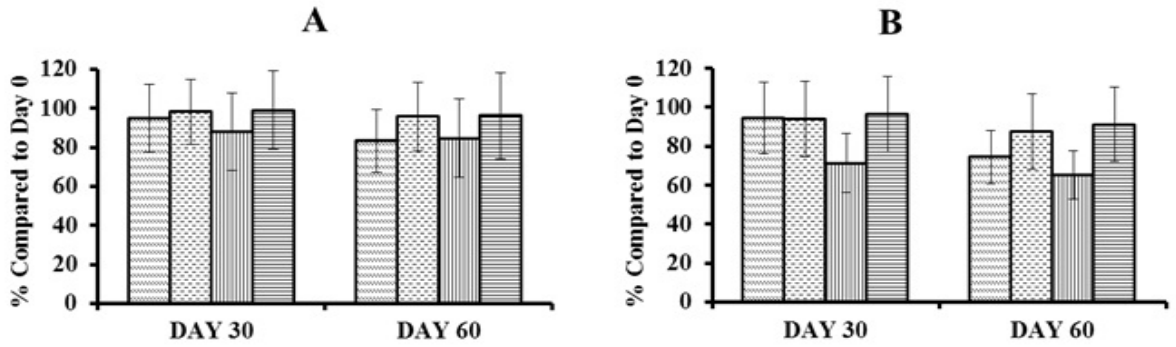

Figure 6: Stability data of phase solubility solutions of zaleplon at A. 25°C/60%RH and B. 40°C/75%RH on day 30 and 60 with BCD (```), HP-β-CD (----),SBE- β-CD (||||), Crysmeb(≡) estimating for % complexation efficiency with respect to day 0.

HP-β-CD-SD) demonstrated the superior release of drug from lyo and SD formulations. This may be due to enhanced wettability influenced by complex formation and reduced drug crystallinity [21] (Figure 5). Increased Q_5 and Q_{30} values for all formulations and physical mixtures over pure drug suggest CD's capacity to enhance the zaleplon's solubility (Table 4). However, the release from of spray-dried formulations observed to be extremely higher (Zal-Crys-SD (101.97±6.41) and Zal-HP-β-CD-SD (97.18±4.21)) within 30 minutes. The higher release may be due to the formation of amorphous particulate powder with increased surface area. Both SD and Lyo solid dispersions resulted in elevated initial dissolution rate (IDR) over the pure drug (3.46±0.12) which may be due to the presence of stable amorphous complexes (Table 4). The dissolution efficiency values of Lyo solid dispersions (34.66±2.41 and 45.51±1.26) and SD solid dispersions (51.74±4.91 and 45.67±4.01) were much higher than that of pure drug (26.48±3.36) (Table 4). The following factors might have individually or in combination improved the dissolution rate and efficiency of zaleplon; (1) CD surfactant behavior has an ameliorating impact on the dissolution of drug by impeding the interfacial tension between drug and dissolution medium; (2) formation of complexes will favor the solubility of drug (determined by phase solubility); (3) transformation of crystalline drug to amorphous complexes in solid state thus elevating the solubility of guest molecule; (4) higher stability of formed inclusion complexes by lyophilization and/or spray drying [29]. The smaller mean dissolution time values of drug from Lyo (3.75±0.53 and 3.97±1.01) and SD (6.09±0.84 and 5.71±1.03) solid dispersions with Crysmeb and HP-β-CD over pure drug (16.71±1.02) suggest the formation of *in situ* molecular

inclusion complexes instantaneously in dissolution media [6] (Table 4). Further, the rapid formation of inclusion complexes can be predicted from higher values of mean drug dissolution rate from all formulations with respect to drug alone. Physical mixtures have shown improved dissolution parameters due to tendency of CD to form *in situ* inclusion complexes in aqueous media. Overall, a 1.8 fold and 2.08 fold of enhancement in the dissolution of drug from lyophilized and spray dried complexes with Crysmeb and HP-β-CD reveals the ability of cyclodextrins in enhancing the dissolution rate of poorly soluble drug zaleplon.

Stability studies

The results obtained from stability studies are depicted in Figure 6. There was no change in the CE values with respect to day 0 in the aqueous CD solutions of β-CD, Crysmeb and HP-β-CD by the end of 60th day demonstrating the higher stability of inclusion complexes at 25°C and 40°C. The instability of inclusion complexes formed by SBE-β-CD attributes to the steric interactions between host and guest molecule along with deprived hydrophobic factors, which controls the penetration of lipophilic actives to spacious cavity of CD [30].

Conclusion

The possibility of stable molecular inclusion complexes with β-CD, Crysmeb and HP-β-CD is attributed to the superior solubility of drug as per phase solubility studies. Solid dispersions processed by Lyo and SD technique have revealed formation of amorphous stable complexes with enhanced dissolution rate, which may further influence the bioavailability of zaleplon. Solid-state characterization studies confirmed the formation of amorphous powder formulations. The enhancement of drug dissolution efficiency from CD solid dispersions ensure Crysmeb and HP-β-CD ability as potential carries to enhance the oral delivery of zaleplon. However, further investigations in animal models are required to confirm the ability of these carriers to improve bioavailability of zaleplon.

Acknowledgements

The authors would like to thank Symed Labs and Maven life Sciences for generously providing drug samples.

References

1. Lipinski CA. Poor aqueous solubility-an industry wide problem in ADME screening. American Pharmaceutical Review. 2002; 5:82-5.
2. Burcham D, Maurin M, Hausner E, Huang SM. Improved oral bioavailability of the hypocholesterolemic DMP 565 in dogs following oral dosing in oil and glycol solutions. Biopharm Drug Dispos. 1997; 18(8):737-42.
3. Šentjurc M, Vrhovnik K, Kristl J. Liposomes as a topical delivery system: the role of size on transport studied by the EPR imaging method. J Control Release. 1999; 59(1):87-97.
4. Rogers TL, Hu J, Yu Z, Johnston KP, Williams RO. A novel particle engineering technology: spray-freezing into liquid. Int J Pharm. 2002; 242(1-2):93-100.
5. Köllmer M, Popescu C, Manda P, Zhou L, Gemeinhart RA. Stability of Benzocaine Formulated in Commercial Oral Disintegrating Tablet Platforms. AAPS PharmSciTech. 2013; 14(4):1333-40. doi: 10.1208/s12249-013-0015-5.
6. Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J Pharm Sci. 1996; 85(10):1017-25.
7. Lyra MA, Soares-Sobrinho JL, Figueiredo RC, Sandes JM, Lima ÁA, Tenório RP, et al. Study of benznidazole–cyclodextrin inclusion complexes, cytotoxicity and trypanocidal activity. Journal of Inclusion Phenomena and Macrocyclic Chemistry. 2012; 73(1-4):397-404. doi: 10.1007/s10847-011-0077-5.
8. Stella VJ, He Q. Cyclodextrins. Toxicol Pathol. 2008; 36(1):30-42. doi: 10.1177/0192623307310945.
9. De Paula D, Oliveira DCR, Tedesco AC, Bentley MVLB. Enhancing effect of modified beta-cyclodextrins on in vitro skin permeation of estradiol. Revista Brasileira de Ciências Farmacêuticas. 2007; 43(1):111-20. doi: http://dx.doi.org/10.1590/S1516-93322007000100014.
10. Rasheed A, Kumar A, Sravanthi V. Cyclodextrins as drug carrier molecule: a review. Sci Pharm. 2008; 76:567-98. doi: 10.3797/scipharm.0808-05.
11. Drover DR. Comparative pharmacokinetics and pharmacodynamics of short-acting hypnosedatives: zaleplon, zolpidem and zopiclone. Clin Pharmacokinet. 2004; 43(4):227-38.
12. Dooley M, Plosker GL. Zaleplon: a review of its use in the treatment of insomnia. Drugs. 2000; 60(2):413-45.
13. Janga KY, Jukanti R, Sunkavalli S, Velpula A, Bandari S, Kandadi P, et al. In situ absorption and relative bioavailability studies of zaleplon loaded self-nanoemulsifying powders. J Microencapsul. 2013; 30(2):161-72. doi: 10.3109/02652048.2012.714408.
14. Janga KY, Jukanti R, Velpula A, Sunkavalli S, Bandari S, Kandadi P, et al. Bioavailability enhancement of zaleplon via proliposomes: Role of surface charge. Eur J Pharm Biopharm. 2012; 80(2):347-57. doi: 10.1016/j.ejpb.2011.10.010.
15. Higuchi T, Connors KA. Phase-solubility techniques. Adv Anal Chem Instrum. 1965; 4(2):117-212.
16. De Waard H, Hinrichs W, Visser M, Bologna C, Frijlink H. Unexpected differences in dissolution behavior of tablets prepared from solid dispersions with a surfactant physically mixed or incorporated. Int J Pharm. 2008; 349(1-2):66-73.
17. Malladi M, Jukanti R, Nair R, Wagh S, Padakanti H, Mateti A. Design and evaluation of taste masked dextromethorphan hydrobromide oral disintegrating tablets. Acta Pharm. 2010; 60(3):267-80. doi: 10.2478/v10007-010-0025-8.
18. Al-Hamidi H, Edwards AA, Mohammad MA, Nokhodchi A. To enhance dissolution rate of poorly water-soluble drugs: glucosamine hydrochloride as a potential carrier in solid dispersion formulations. Colloids Surf B Biointerfaces. 2010; 76(1):170-8. doi: 10.1016/j.colsurfb.2009.10.030.
19. Valleri M, Mura P, Maestrelli F, Cirri M, Ballerini R. Development and evaluation of glyburide fast dissolving tablets using solid dispersion technique. Drug Dev Ind Pharm. 2004; 30(5):525-34.
20. Chadha R, Arora P, Gupta S, Jain DS. Complexation of nevirapine with β-cyclodextrins in the presence and absence of Tween 80: characterization, thermodynamic parameters, and permeability flux. Journal of thermal analysis and calorimetry. 2011; 105(3):1049-59. doi: 10.1007/s10973-011-1406-z.
21. Freitas MRd, Rolim LA, Soares MFdLR, Rolim-Neto PJ, Albuquerque MMd, Soares-Sobrinho JL. Inclusion complex of methyl-β-cyclodextrin and olanzapine as potential drug delivery system for schizophrenia. Carbohydr Polym. 2012; 89(4):1095-100. doi: 10.1016/j.carbpol.2012.03.072.
22. Zeng J, Ren Y, Zhou C, Yu S, Chen WH. Preparation and physicochemical characteristics of the complex of edaravone with hydroxypropyl-β-cyclodextrin. Carbohydrate Polymers. 2011; 83(3):1101-5. doi:10.1016/j.carbpol.2010.09.007.

23. Nagarsenker M, Londhe VY, Nadkarni G. Preparation and evaluation of liposomal formulations of tropicamide for ocular delivery. Int J Pharm. 1999; 190(1):63-71.

24. Pralhad T, Rajendrakumar K. Study of freeze-dried quercetin–cyclodextrin binary systems by DSC, FT-IR, X-ray diffraction and SEM analysis. Journal of pharmaceutical and biomedical analysis. 2004; 34(2):333-9. doi: 10.1016/S0731-7085(03)00529-6.

25. Schneider HJ, Hacket F, Rüdiger V, Ikeda H. NMR studies of cyclodextrins and cyclodextrin complexes. Chem Rev. 1998; 98(5):1755-86.

26. Fernandes CM, Carvalho RA, da Costa SP, Veiga FJ. Multimodal molecular encapsulation of nicardipine hydrochloride by β-cyclodextrin, hydroxypropyl-β-cyclodextrin and triacetyl-β-cyclodextrin in solution. Structural studies by 1 H NMR and ROESY experiments. Eur J Pharm Sci. 2003; 18(5):285-96.

27. Ali SM, Asmat F, Maheshwari A, Koketsu M. Complexation of fluoxetine hydrochloride with β-cyclodextrin. A proton magnetic resonance study in aqueous solution. Il Farmaco. 2005; 60(5):445-9. doi:10.1016/j.farmac.2005.03.003.

28. Bergeron R, Rowan R. The molecular disposition of sodium p-nitrophenolate in the cavities of cycloheptaamylose and cyclohexaamylose in solution. Bioorganic Chemistry. 1976; 5(4):425-36. doi:10.1016/0045-2068(76)90027-4.

29. Mura P, Faucci MT, Bettinetti GP. The influence of polyvinylpyrrolidone on naproxen complexation with hydroxypropyl-beta-cyclodextrin. Eur J Pharm Sci. 2001; 13(2):187-94.

30. Loftsson T, Hreinsdóttir D, Masson M. Evaluation of cyclodextrin solubilization of drugs. International journal of pharmaceutics. 2005; 302(1-2):18-28. doi:10.1016/j.ijpharm.2005.05.042.