The Improvement of Lorazepam Solubility by Cosolvency, Micellization and Complexation

Jasmina Hadžiabdić[1]*, Alisa Elezović[1], Belma Imamović[2] and Ervina Bečić[2]✉

[1] Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Sarajevo, Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina.
[2] Department of Control of Drug, Faculty of Pharmacy, University of Sarajevo, Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina.

ABSTRACT

Lorazepam is an almost water insoluble substance. This study investigated the solubilization of lorazepam depending on media pH, cosolvents (ethanol, propylene glycol, polyethylene glycol 200 and 400), surfactants (Tween 80, Tween 20, Brij 35, sodium-cholate, sodium-deoxycholate, sodium-taurocholate) and cyclodextrins (α-cyclodextrin, β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin). The main objective was to find the most suitable method for providing good solubility of this drug and thus its formulation in a liquid dosage form.

Based on the results, the changes in the pH value of the media do not lead to a greater solubility of lorazepam. Of the cosolvents used, the greatest increase in solubility of lorazepam in water was achieved with ethanol. Of the bile salts used, sodium taurocholate showed the best solubilization ability, while Brij 35 was the best of the non-ionic surfactants. The solubility of lorazepam with the cyclodextrin derivative, 2-hydroxypropyl-β-cyclodextrin was better than natural cyclodextrin. Surfactants have the highest ability of solubilization of lorazepam in water.

Keywords: solubility enhancement, lorazepam, pH, cosolvents, surfactants, cyclodextrins.

INTRODUCTION

Lorazepam has anxiolytic, sedative, hypnotic, and anticonvulsant as well as muscle relaxant properties. Chemically, it is 7-chloro-5-(2-chlorophenyl)-1, 3-dihydro-3-hydroxy-2H-1,4-benzodiazepine-2-on, having a formula $C_{15}H_{10}C_{l2}N_2O_2$ (Figure 1). It is a poor water soluble substance. The aqueous solubility of lorazepam is 0.0485 mg/ml (1.51×10^{-4} mol/L) [1].

In clinical practice, a non-aqueous solution of lorazepam is intended for parenteral administration. Often, in a certain volume of water, an adequate concentration of the drug cannot be achieved during formulation of a liquid dosage form due to the low solubility of the drug. In practice, different methods are often used for improving the solubility of slightly soluble or practically insoluble substances in water. The four most commonly used techniques for solubilization of drugs for parenteral use are: adjustment of pH, addition of a cosolvent, addition of a surfactant, or complexation. The chosen method of solubilization of a drug is dependent upon how efficiently the drug can be solubilized and upon the biocompatibility of the vehicle. Commonly different methods should be combined for solubility enhancement of the drugs. Cosolvents (ethanol, propylene glycol, polyethylene glycol 200 and 400), surfactants [polyoxyethylene sorbitan monooleate (Tween 80), polyoxyethylene sorbitan monolaurate (Tween 20), polyoxyethylene lauryl ether (Brij 35), sodium cholate, sodium deoxycholate, sodium taurocholate)] and complexation agents [cyclodextrins: α-

Received on 9/3/2011 and Accepted for Publication on 2/1/2012.
✉ E-mail: hadziabdicjasmina@yahoo.com

cyclodextrin (α-CD), β-cyclodextrin (β-CD) and 2-hydroxypropyl-β-cyclodextrin (2-HP-βCD)] were used as solubilizing agents in this study. Cosolvents, surfactants and cyclodextrins were used as additional substances in order to increase the solubility of lorazepam. [4]

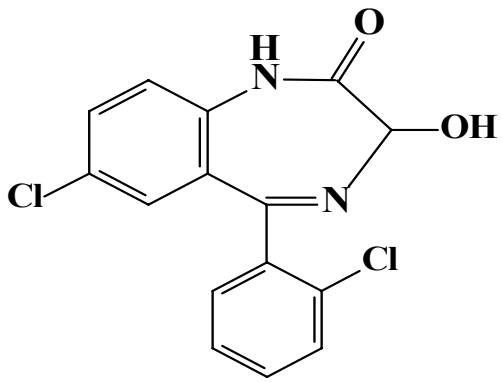

Figure 1: Structure of Lorazepam.

Cosolvents are organic compounds that are substantially miscible with water. Cosolvents have small hydrocarbon regions. Since these regions are nonpolar and they do not interact strongly with water, they can reduce the ability of the aqueous system to squeeze out nonpolar solutes. [5] The new system of solvents was formed by mixing cosolvents and water. Increasing amounts of cosolvent enhance the solubility of lorazepam, caused by stronger salvation affinity of these less polar molecules. [4, 6]

Micellar solubilization is a powerful alternative for dissolving a hydrophobic drug in aqueous environments. Organic solutes can be solubilized by incorporation into the micelles formed by surfactants. The more nonpolar the solute, the more likely it is to be incorporated near the core or center of the micelle. [4, 5, 7]

Cyclodextrins (CDs) are cyclic oligosaccharides containing at least 6 D-(+)-glucopyranose units attached by α-(1,4) glucoside bonds. The most important structural feature of cyclodextrins is a hydrophobic central pocket with hydrophilic outer surface. CDs are capable of forming an inclusion complex with a small molecule in a host guest fashion, which in turn affects many properties of the guest molecule, most important among them being solubility enhancement. [8, 9] With the introduction of the hydrophilic derivatives of β-cyclodextrin such as hydroxypropyl-β-cyclodextrin (HP-β-CD), cyclodextrin complexation has gained more attention in solubilization technology. [10] In order to obtain complexation, the compounds have to bind a complex first in solution. These inclusion complexes exist both in an aqueous solution and in a solid state.

This study investigated the use of the traditional solubilization approaches to increase the solubility of lorazepam. Our aim was to investigate the solubility of lorazepam in phosphate buffer solutions, in water/cosolvents mixtures, and in water/surfactants mixtures as well as its solubility in water/cyclodextrins mixtures. Comparisons of the different methods of solubility improvement, in particular, investigations in the inclusion reaction in different aqueous solvent mixtures were performed.

EXPERIMENTAL

Materials

The following materials were used: Lorazepam (Sigma-Aldrich, Germany); Acidum sulfuricum concentratum, 95-97%, pro analysi, (Kemika, Croatia); Methanolum, pro analysi, (Kemika, Croatia); Acidum hydrochloricum fumans, 37%, pro analysi, (Alkaloid, Macedonia); Disodium hydrogen phosphate (Alkaloid, Macedonia); Potassium dihydrogen phosphate (Alkaloid, Macedonia); Acidum phosphoricum concentratum, 85%, pro analysi, (Fluka, Chemika, Switzerland); aethanolum concentratum (Sigma-Aldrich, Germany); Polyethylene glycol 200 (Macrogol 200) (Sigma-Aldrich, Germany); Polyethylene glycol 400 (Macrogol 400) (Sigma-Aldrich, Germany); 1,2–Propanediol (Propylene glycol) (Sigma-Aldrich, Germany); Polysorbate 20 (polyoxyethylene sorbitan monolaurate) (Merck, Germany); Polysorbate 80 (polyoxyethylene sorbitan monooleate) (Merck, Germany); Brij 35 (Polyoxyethylene lauryl ether) (Merck, Germany); Sodium cholate (Fluka, Chemika, Switzerland); Sodium deoxycholate (Fluka, Chemika, Switzerland); Sodium taurocholate (Fluka, Chemika,

Switzerland); α-Cyclodextrin (Fluka, Chemika, Switzerland); β-Cyclodextrin (Fluka, Chemika, Switzerland); 2-Hydroxypropyl-β-Cyclodextrin (Fluka, Chemika, Switzerland). All the employed reagents were of analytical grade.

Solubility Studies

The solubility of lorazepam was estimated by the solubility method of Higuchi and Connors.[11] Solubility measurements and the determination of saturation concentrations were carried out by adding excess amounts of lorazepam to phosphate buffer solutions, water/cosolvent mixtures, water/surfactants mixtures and water/cyclodextrins mixtures. Solubility of the investigated substance was determined in phosphate buffer solutions with pH values from 2.0 to 8.0. Solutions with cosolvents were prepared at concentrations of 1 to 60 % (w/w). Solutions with anionic surfactants were prepared at concentrations from 1 to 20 % (w/w), while solutions with non-ionic surfactants were prepared at concentrations from 1 to 35 % (w/w). Saturated solutions of cyclodextrins were prepared and used for the solubility measurements as well as for the determination of the stability constants.

The lorazepam powder was added to vials containing already mentioned percentages of cosolvents (Table 2), surfactants (Table 4 and 5) or complexation agents (Table 8). Triplicate sample vials were prepared for each solubilizing agent at its particular concentration and were placed on the mechanical rotator, protected from light, at room temperature for 24 hours, until equilibrium was reached. Undissolved substances were visualized before and after the achievement of equilibrium. [11, 12]

Samples were filtered through a 0.2 μm pore size membrane filter (Cellulose acetate filter, Sartorius, Germany). The concentrations of dissolved lorazepam in phosphate buffer solutions, water/cosolvent mixtures, water/surfactants mixtures and water/cyclodextrins mixtures were determined by absorption spectroscopy using a Shimadzu UV-1601, UV-VIS spectrophotometer (Shimadzu, Japan) at 230 nm, in 0.1 mol/L HCl **(**calibration curve; concentration range: 1.5 – 6.0 mgL^{-1}, correlation coefficient $R^2 = 0.9999$). All experiments were carried out in triplicate.

RESULTS AND DISCUSSION

Lorazepam Solubility in Phosphate Buffer Solutions

Phosphate buffer solutions of lorazepam were diluted with hydrochloric acid (0.1.mol/L) since the sample of lorazepam in a phosphate buffer solution is opaque when in contact with ethanol [13].

The results for lorazepam solubility by changing the pH values of phosphate buffer solutions are summarized in Figure 2 and Table 1. Table 1 shows the change of lorazepam solubility in phosphate buffer solutions with pH values from 2.0 to 8.0, compared to its solubility in water.

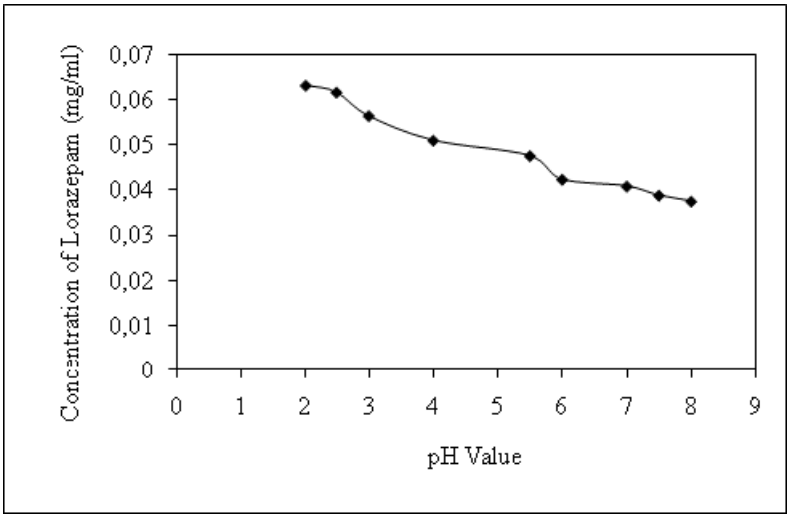

Figure 2: Solubility change of lorazepam with regard to the pH values of phosphate buffer solutions.

Table 1: Influence of pH values on solubility of lorazepam at 22 ± 2 oC.

pH value of phosphate buffer solutions	2.0	2.5	3.0	4.0	5.5	6.0	7.0	7.5	8.0
Solubility enhancement of lorazepam (fold)	1.30	1.27	1.16	1.05	0.98	0.87	0.84	0.80	0.77

The investigated lorazepam is a highly lipophilic compound as seen from its solubility ($S_{o(lorazepam)}$ = 0.0485 mg/ml of water and partition coefficient $logP_{(lorazepam)}$ = 2.36 when measured at 20 ^{o}C). It is an amphiphilic substance. Lorazepam has two pKa values (1.3 and 11.5) determined spectrophotometrically in aqueous buffers. Based on the results, it was found that lorazepam solubility was decreased with an increasing pH. When the pH value is lower, the percentage of protonation of lorazepam is higher, but this is not as pronounced as with the other benzodiazepines (diazepam, nitrazepam) while the percentage of protonation in an alkaline medium is decreased. Protonation at low pH occurs at the nitrogen atom in position 4. Deprotonation occurs at a high pH with the loss of the hydrogen atom from the 3-hydroxyl group. [14]

Lorazepam Solubility in Various Mixtures with Cosolvents

The solubility values of lorazepam as functions of the cosolvent concentrations [propylene glycol (PG), polyethylene glycol 200 (PEG 200), polyethylene glycol 400 (PEG 400), ethanol (EtOH)] are presented in Figure 3.

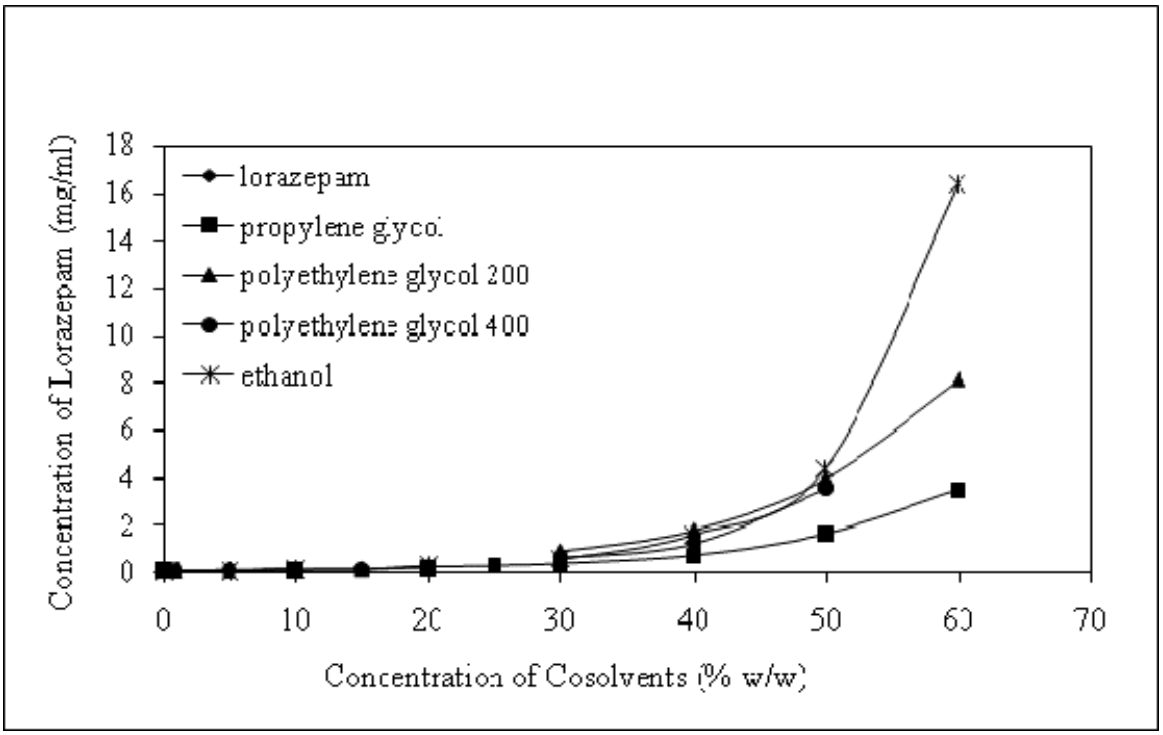

Figure 3: Lorazepam solubility in aqueous solutions depending on the concentration of cosolvents.

A new system of solvents was formed by mixing cosolvents and water. The cosolvents were water miscible

solvents, widely used in the pharmaceutical field for solubilization purpose. Their dielectric constants (ε) are lower than that of water (ε = 78.36), so they are better for dissolving a poorly soluble drug substance. [4, 6] Dielectric constant (ε) of 60 % ethanol was 45.92, while ε of 60 % propylene glycol was 50.54. The dielectric constant for 30 % ethanol was 62.14 while ε of 30 % propylene glycol was 64.45. [15] Dielectric constants of the solvent mixtures were calculated from the relation $\varepsilon_{mix} = \varepsilon_{ws} f_{ws} + \varepsilon_{\varepsilon ss} f_{ss}$, where ε and *f* are the dielectric constant and volume fraction, respectively and subscripts mix, ws and ss represent values for the mixture, weaker solvent and stronger solvent, respectively. The polarity of a cosolvent is not the only factor affecting the solubility of a drug. The ability of a solvent to form hydrogen bonds with the hetero-atoms in the drug molecule is another important factor governing the solubility of a drug. Similar findings have also been reported by Seedher and Bhatia. [16] The larger σ values of a cosolvent (σ-cosolvent solubilizing power) are more effective in increasing the solubility of lorazepam. The logarithmic relationship between the total drug solubility in a mixed-solvent system and the volume fraction of the stronger solvent can be described by Equation 1. [6]

$$\log[S_{tot}] = \log[S_u] + \sigma[C] \quad (1)$$

where $[S_{tot}]$ is the total apparent drug solubility, $[S_u]$ is the solubility of the drug in water, [C] is the volume fraction of the cosolvent and σ is the cosolvent solubilizing power of the solute. The σ value was obtained from the linear $\log[S_{tot}]$ versus [C] plots. The order of solubilizing power of the cosolvents is EtOH > PEG 400 > PEG 200 > PG ($\sigma_{(ethanol)} = 4.1$, $\sigma_{(polyethylene\ glycol\ 400)} = 3.8$, $\sigma_{(polyethylene\ glycol\ 200)} = 3.7$ and $\sigma_{(propylene\ glycol)} = 2.7$) [4]. The σ value depends on the polarity of both the solvent and the solute. The more nonpolar the solvent and the solute, the larger the σ value. The lesser polar solvents were found to increase solubility by a greater extent, thus accentuating the hydrophobic interaction mechanism.

The aim was to determine the concentration of a particular cosolvent that could produce a dissolution of lorazepam in water. The lorazepam powder was added to vials containing cosolvents in the concentrations listed in Table 2. Lorazepam solubility was not assessed in solutions that contained 1.0%, 15.0% and 25.0% of ethanol, 25.0% of polyethylene glycol 400, 25.0% of polyethylene glycol 200 and 1.0 and 15.0% of propylene glycol. The concentration of a cosolvent was the one that relates to those of the drugs in water solutions. Table 2 shows the increase in lorazepam solubility in aqueous solutions of chosen cosolvents, in comparison to their solubility in water. [17]

Table 2: Influence of cosolvent concentration on solubility of lorazepam at 22 ± 2 oC.

Cosolvents	**Solubility enhancement of lorazepam (fold)**									
	1%	5%	10%	15%	20%	25%	30%	40%	50%	60%
EtOH	ND	1.51	2.91	ND	6.25	ND	11.40	32.99	89.69	337.73
PEG 400	1.20	1.65	2.27	2.41	5.44	ND	18.04	36.08	80.93	167.01
PEG 200	1.18	1.24	1.81	2.27	3.88	ND	12.47	24.49	72.68	107.63
PG	ND	1.79	3.56	ND	4.60	5.36	6.49	13.51	32.47	71.65

ND = not determined

Lorazepam is mainly used in different dosage forms as an active substance. In its medical form, lorazepam is commonly available as tablets and as a solution for injection. The injectable solution comes in 1ml ampoules containing 2 mg or 4 mg lorazepam. To prepare the liquid form (aqueous solution) of lorazepam containing 2 mg of lorazepam/ml, it is necessary to have more than 50 % (w/w) of propylene glycol, or more than 40% (w/w) of polyethylene glycol 400, polyethylene glycol 200 or ethanol. To completely dissolve 4 mg/ml lorazepam in

water more than 50 % (w/w) of polyethylene glycol 400 or polyethylene glycol 200 or less than 50 % (w/w) of ethanol is needed (see Table 3). It is clearly evident that lorazepam molecules preferably solubilize in a nonpolar environment rather than polar (aqueous) surroundings.

Table 3: Lorazepam solubility in different concentrations of cosolvents.

Cosolvents	Lorazepam solubility (mg/ml)									
	1%	5%	10%	15%	20%	25%	30%	40%	50%	60%
EtOH	ND	0.073	0.141	ND	0.303	ND	0.553	1.600	4.350	16.38
PEG 400	0.058	0.080	0.110	0.117	0.264	ND	0.875	1.750	3.925	8.100
PEG 200	0.057	0.060	0.088	0.110	0.188	ND	0.605	1.188	3.525	5.220
PG	ND	0.087	0.173	ND	0.223	0.260	0.315	0.655	1.575	3.475

ND = not determined

The use of cosolvents is a technique which is frequently utilized pharmaceutically for the preparation of liquid formulations. The concentration of the solvents that enable these drugs to be solubilized in water is far higher than that allowed for daily intake. When combining two or more cosolvents, better solubility of lorazepam is achieved (the results are not shown here) which also reduces the side effects of the cosolvents than when used singularly.

Influence of the Surfactants on the Solubility of Lorazepam

The solubility of poor water soluble lorazepam was determined in aqueous binary mixtures of sodium-cholate, sodium-deoxycholate, and sodium-taurocholate from 1 % to 20 % surfactant concentrations. The determination of lorazepam solubility in bile salt solutions (in solutions of sodium-cholate, sodium-deoxycholate and sodium-taurocholate) was carried out in 0.5 % w/v H_2SO_4/CH_3OH, as a solvent for the dilution of an aqueous lorazepam solution. These tested solutions when in contact with 0.1 mol/L HCl create a fuzzy sediment. Figure 4 shows the effect of sodium-cholate, sodium-deoxycholate and sodium-taurocholate on lorazepam solubility.

The results of the changes in lorazepam solubility in water, depending on the increase of Tween 80, Tween 20 and Brij 35 concentrations are shown in Figure 5.

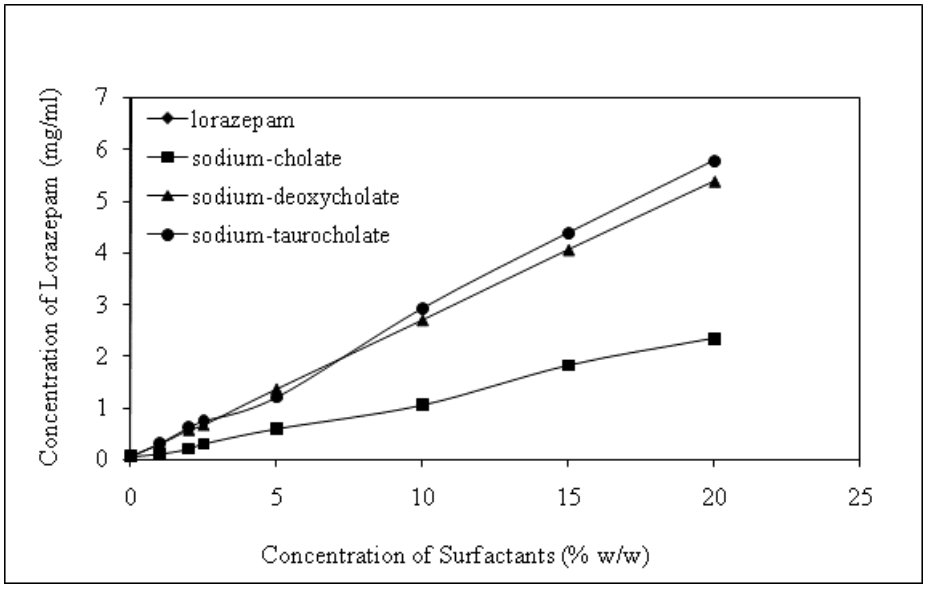

Figure 4: The increase of lorazepam solubility in the presence of bile salts.

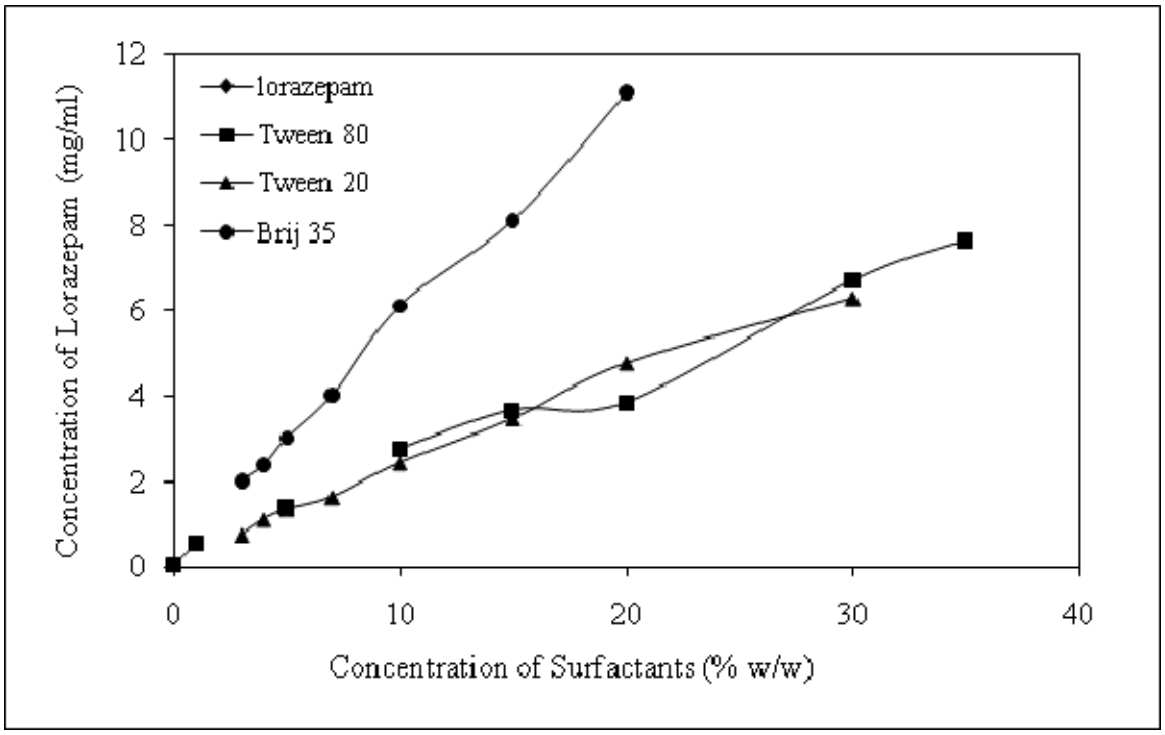

Figure 5: Concentration of dissolved lorazepam at various concentrations of non-ionic surfactants.

An increase in the solubility of practically insoluble lorazepam was achieved using anionic surfactants (sodium-cholate, sodium-deoxycholate, sodium-taurocholate) and non-ionic surfactants (Brij 35, Tween 20 and Tween 80) . [18] As shown in Figures 4 and 5, the lorazepam solubility increased linearly with the concentration of surfactants. The best solubility was achieved with sodium-taurocholate, of all the tested bile salts, while the best lorazepam solubility in water was achieved with Brij 35, of all the tested non-ionic surfactants. [19] Brij 35 (in concentrations of 3-20 % w/w) increased the solubility of lorazepam by 42.01 to 229.38 fold, Tween 20 (3-30 % w/w) increased the solubility by 15.57 to 128.87 fold and Tween 80 (1-35 % w/w) increased lorazepam solubility by 12.01 to 157.73 fold. Table 4 and 5 show the increase of lorazepam solubility in aqueous solutions of anionic and non-ionic surfactants, compared to its solubility in water. [17]

Table 4: Influence of anionic surfactant concentration on lorazepam solubility at 22 ± 2 oC.

Anionic surfactants	Solubility enhancement of lorazepam (fold)						
	1.0%	2.0%	2.5%	5%	10%	15%	20%
Sodium-cholate	2.06	3.92	5.98	11.96	21.44	37.32	48.25
Sodium-deoxycholate	5.98	11.55	13.61	27.84	55.46	83.51	110.82
Sodium-taurocholate	6.19	12.58	15.05	24.54	60.00	89.90	119.18

Table 5: Influence of non-ionic surfactant concentration on lorazepam solubility at 22 ± 2 oC.

Non-ionic surfactants	Solubility enhancement of lorazepam (fold)										
	1.0%	2.0%	3.0%	4.0%	5.0%	7.0%	10.0%	15.0%	20.0%	30.0%	35.0%
Tween 20	ND	ND	15.57	22.89	27.84	33.76	50.26	71.65	98.45	128.87	ND
Tween 80	12.01	ND	ND	ND	29.25	ND	57.21	75.77	79.38	138.14	157.73
Brij 35	ND	ND	42.01	49.23	62.37	82.29	126.29	167.52	229.38	ND	ND

ND = not determined

To dissolve 2 mg/ml of lorazepam in water, more than 15 % of sodium-cholate, more than 5 % of sodium-deoxycholate, or less than 10 % of sodium-taurocholate concentrations are needed (see Table 6). To make a lorazepam concentration in liquid form of 2 mg lorazepam / ml of water, more than 5 % of Tween 80, more than 7 % of Tween 20, or less than 3 % Brij 35 is needed (see Table 7). To dissolve 4 mg /ml of lorazepam in water, more than 20 % of sodium-cholate, about 15 % of sodium-deoxycholate or less than 15 % of sodium-taurocholate is needed (see Table 6). To dissolve 4 mg of lorazepam / ml of water, 20% of Tween 80, more than 15 % of Tween 20 or less than 7 % Brij 35 should be used (see Table 7).

Table 6 Lorazepam solubility in different concentrations of anionic surfactants.

Anionic surfactants	Lorazepam solubility (mg/ml)						
	1.0%	2.0%	2.5%	5%	10%	15%	20%
Sodium-cholate	0.10	0.19	0.29	0.58	1.04	1.81	2.34
Sodium-deoxycholate	0.29	0.56	0.66	1.35	2.69	4.05	5.36
Sodium-taurocholate	0.30	0.61	0.73	1.19	2.91	4.36	5.78

Table 7. Lorazepam solubility in different concentrations of non-ionic surfactants.

Non-ionic surfactants	Lorazepam solubility (mg/ml)										
	1.0%	2.0%	3.0%	4.0%	5.0%	7.0%	10.0%	15.0%	20.0%	30.0%	35.0%
Tween 20	ND	ND	0.76	1.11	1.35	1.64	2.44	3.48	4.78	6.25	ND
Tween 80	0.58	ND	ND	ND	1.42	ND	2.78	3.68	3.85	6.70	7.65
Brij 35	ND	ND	2.04	2.39	3.03	4.03	6.13	8.13	11.13	ND	ND

ND = not determined

It was found that surfactants with higher HLB values were better solubilizers ($HLB_{(Tween\ 80)}$ = 15, $HLB_{(Tween\ 20)}$ = 16.7, $HLB_{(Brij\ 35)}$ = 16.9). Solubilization capacity was found to increase with an increase in the hydrocarbon chain length of the surfactant, suggesting that the hydrocarbon core of micelles is the locus of solubilization.

Solubility Enhancement of Lorazepam by Complexation with Cyclodextrins

The result of the change in lorazepam solubility in water depending on α-cyclodextrin (α-CD), β-cyclodextrin (β-CD) and 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD) concentrations are shown in Figures 6, 7 and 8.

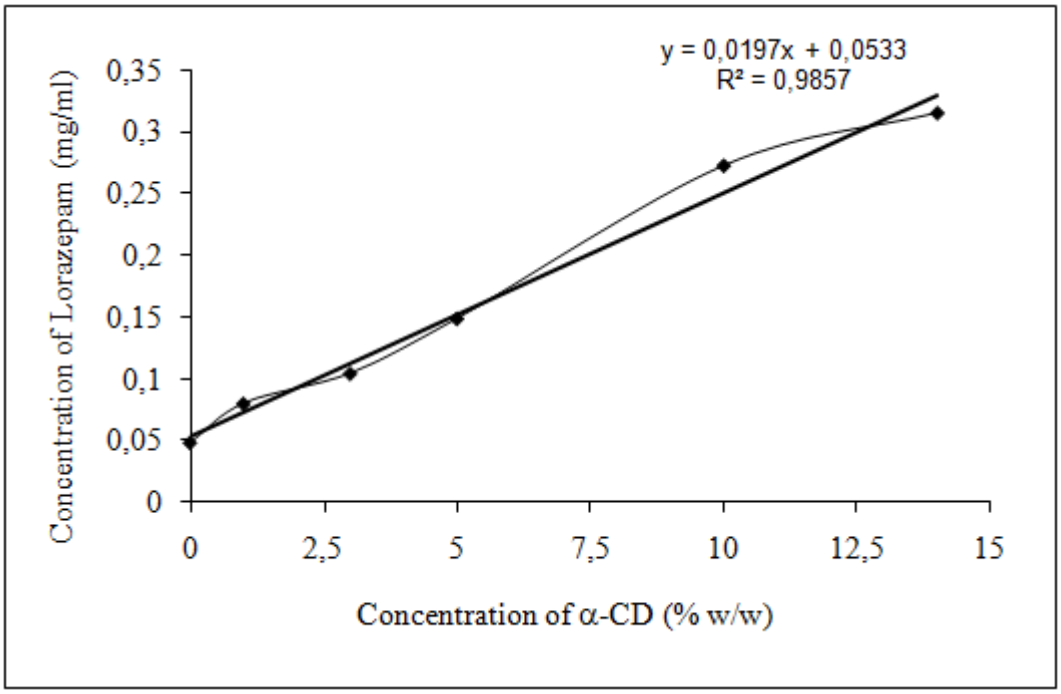

Figure 6: Phase solubility diagram of lorazepam and α -cyclodextrin in distilled water at room temperature.

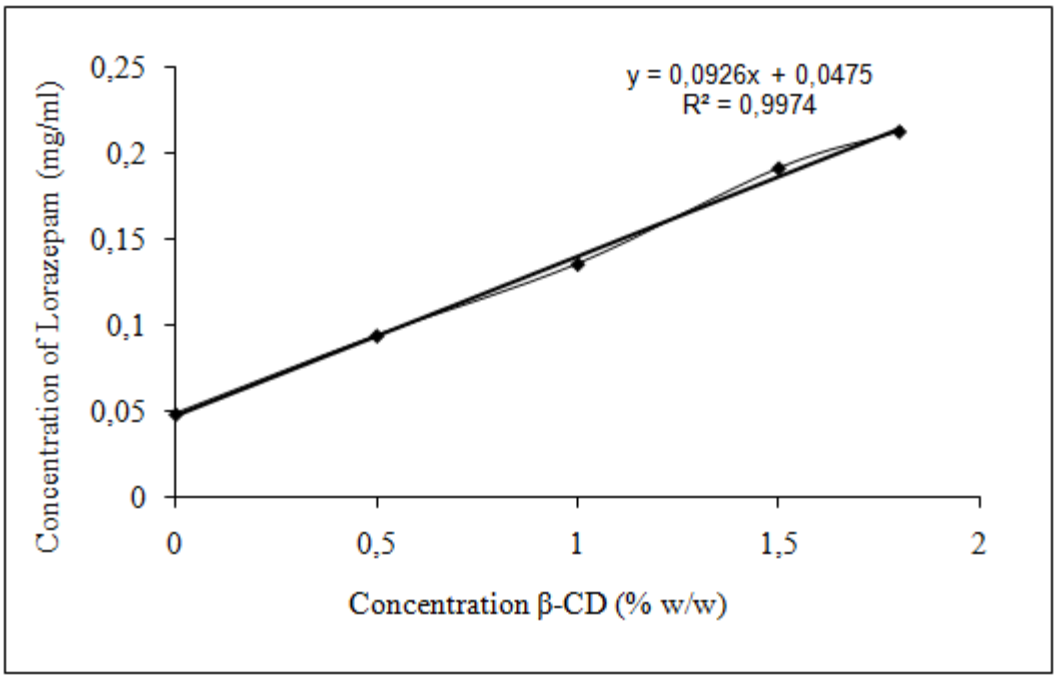

Figure 7: Phase solubility diagram of lorazepam and β-cyclodextrin in distilled water at room temperature.

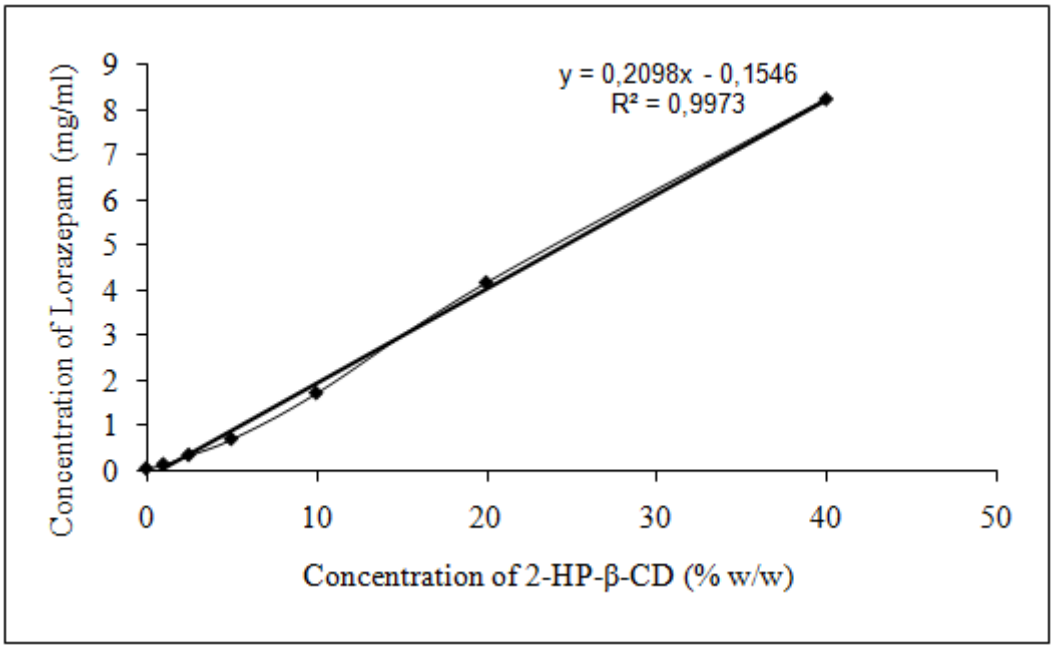

Figure 8: Phase solubility diagram of lorazepam and 2-hydroxypropyl-β-cyclodextrin in distilled water at room temperature.

Each point on the graphs is the arithmetic mean of three determinations.

The aim of this work was to investigate the solubility properties of lorazepam with α- and β-cyclodextrin (Figures 6 and 7) and with 2-hydroxypropyl-β-cyclodextrin, as modified cyclodextrin (Figure 8). Concentrations of these cyclodextrins were selected based on their solubility in water. Solubility of α-cyclodextrin is 14.5 %. Testing of lorazepam solubility with the addition of α-cyclodextrin in aqueous solutions was carried out by its addition in concentrations up to 14 %. The testing of lorazepam solubility was carried out in concentrations up to 1.8 % of aqueous solutions of β-cyclodextrin. The natural cyclodextrins, in particular β-cyclodextrin, have limited aqueous solubility and their complex formation with lipophilic drugs often results in precipitation of a solid drug-cyclodextrin complex. [20] Thus, the solubility of β-cyclodextrin in water is only about 1.85 % at room temperature (15.86 mM in case of β-CD's solubility limit). This low aqueous solubility is, at least partly, associated with a strong intramolecular hydrogen bonding in the cyclodextrin crystal lattice. Thus, the solubilization of lorazepam with the use of β-cyclodextrin is limited by the low solubility of β-cyclodextrin. [14] Solubility of 2-hydroxypropyl-β-cyclodextrin is greater than 50 % and the testing of lorazepam solubility with the addition of this cyclodextrin in aqueous solutions was carried out by its addition in concentrations up to 40 %.

The solubility of lorazepam is increased 6.5-fold at 14 % of α-CD (0.315 mg/ml of lorazepam), 4.38-fold at 1.8 % β-CD (0.213 mg/ml of lorazepam), and 170.1-fold at 40 % of 2-HP-β-CD (8.25 mg/ml of lorazepam). Table 8 shows the increase of lorazepam solubility in aqueous solutions of chosen cyclodextrins, compared to its solubility in water. [17] Hydroxypropyl-β-cyclodextrin showed a significant increase in lorazepam solubility in water.

Table 8: Influence of cyclodextrin concentration on solubility of lorazepam at 22 ± 2 oC.

Concentration of α-CD (%)	Solubility enhancement of lorazepam (fold)	Concentration of β-CD (%)	Solubility enhancement of lorazepam (fold)	Concentration of 2-HP-β-CD (%)	Solubility enhancement of lorazepam (fold)
1.0	1.65	0.5	1.94	1.0	2.86
3.0	2.15	1.0	2.80	2.5	7.16
5.0	3.06	1.5	3.94	5.0	14.54
10.0	5.62	1.8	4.38	10.0	35.57
14.0	6.50	ND	ND	20.0	86.08
ND	ND	ND	ND	40.0	170.10

ND = not determined

The phase solubility diagrams obtained (Figures 6, 7, 8) are linear and show characteristics of A_L-type of solubility curve. This is attributed to the formation of a soluble complex. A-type phase-solubility profiles are obtained when the solubility of the substrate (i.e. drug) increases with an increasing ligand (i.e. cyclodextrin) concentration. When the complex is first order with respect to ligand and first or higher order with respect to substrate, then A_L-type phase-solubility profiles are obtained. The changes in the solubility of lorazepam resulting from the addition of various concentrations of α-CD, β-CD and 2-HP-β-CD were used to plot phase-solubility diagrams and to evaluate the stoichiometry and stability constant of the resultant complex. The apparent stability constants ($K_{1:1}$) were estimated from the straight line of the phase solubility diagrams according to the following Equation of Higuchi and Connors [11]:

$$K_{1:1} = \frac{slope}{So\ (1 - slope\ )} \qquad (2)$$

where $K_{1:1}$ is the stability constant ($Lmol^{-1}$), S_o (intrinsic solubility) is the saturation concentration of drug (lorazepam) in pure water, and *slope* denotes the slope of the straight line. If the slope is less than one, it is assumed that stoichiometry of these complex is 1:1. Stability constants, calculated using the slope of the curves, are reported in Table 9.

Table 9: Effect of α-cyclodextrin, β-cyclodextrin and 2-HP-β-cyclodextrin on lorazepam intrinsic solubility (S_o), slope of the phase-solubility diagrams, and stability constants ($K_{1:1}$) of binary systems. Values are obtained directly from the drug phase solubility diagram.

	Slope	r^2	$K_{1:1}$ (M^{-1})
lorazepam : α-CD	0.0059	0.9867	39.305
lorazepam : β-CD	0.0327	0.9974	223.878
lorazepam : 2-HP-β-CD	0.0902	0.9973	656.578

In this case, the $K_{1:1}$ values obtained followed the order 2-HP-β-CD > β-CD > α-CD, reflecting the greater affinity of modified cyclodextrin for lorazepam compared with their parent α- and β-CD. It is reported that cyclodextrin-drug complexes with $K_{1:1}$ values in the range of 200-5000 M^{-1} show improved dissolution properties and, hence, better bioavailability. The value of the stability constant ($K_{1:1}$) is used to compare the affinity of a drug for different cyclodextrins or cyclodextrin derivatives. [21]

CONCLUSIONS

This study investigated and compared four different approaches to solubilize lorazepam: pH, cosolvents, surfactants, and cyclodextrins. Solubility enhancement of lorazepam by changing pH value of its aqueous solution has no practical use. Increase of cosolvents, surfactants and cyclodextrins concentration in water leads to lorazepam solubility enhancement. The best solubility of investigated lorazepam was achieved by adding ethanol, polyethylene glycol 400, polyethylene glycol 200 and propylene glycol, respectively. The lorazepam solubility increased linearly with the increase in concentration of surfactants. Sodium-taurocholate, of all the bile salts, showed the best results when used as an agent for solubilization, while Brij 35, as a non-ionic surfactant, showed the best increase of solubility of this group of substances (0.3-5.8 mg/ml in 1-20 % sodium-taurocholate and 2.0-11.1 mg/ml in 3-20 % Brij 35). The increase in solubility displayed concentration dependency on CDs. Among the three CDs used, 2-HP-β-CD showed the highest effects on lorazepam solubility (0.14-8.3 mg/ml in 1-40 % 2-HP-β-CD).

Of all the methods used to increase the solubility of a poor water soluble compound, the micellization should be preferred. The solubility enhancement achieved with the micellization is much higher than the ones achieved by complexation and the cosolvency method or the adjustment of the pH value of the media. Thus, the study generates an array of data for solubilization of lorazepam using various pharmaceutically accepted techniques which could be useful while formulating liquid dosage forms of lorazepam.

REFERENCES

(1) Hadžiabdić J. and Hadžović S. Influence of Particle Size on Water Solubility of Almost Insoluble Benzodiazepines. *Pharmacia.* 2006; 16: 35-39.

(2) Parasrampuria J. In: Encyclopedia of Pharmaceutical Technology. Swarbrick J., Boylan J.C. (Eds); Marcel Dekker Inc., New York, 1994; pp 42-59.

(3) Myrdal P.B. and Yalkowsky S.H. In: Encyclopedia of Pharmaceutical Technology. Swarbrick J., Boylan J.C. (Eds); Marcel Dekker Inc., New York, 1999; pp 161-212.

(4) Babu S.R.P., Subrahmanyam S.V.C., Thimmasetty J., Manavalan R., Valliappan K. and Kedarnath A.S. Solubility Enhancement of Cox-II Inhibitors by Cosolvency Approach. *J. Pharm. Sci.* 2008; 7 (2): 192-126.

(5) Corrigan O.I. and Healy A.M. In: Encyclopedia of Pharmaceutical Technology. Swarbrick J. (Ed); Informa Healthcare USA, 2007; pp 3583-3588.

(6) Yalkowsky S.H. and Rubino J.T. Solubilization of cosolvents 1: organic solutes in propylene glycol- water mixture. *J. Pharm. Sci.* 1985; 74 (4): 416-421.

(7) Florence A.T. and Attwood D. In: Physicochemical principles of pharmacy. Pharmaceutical Press, London, Great Britain, 2006; Chapter 6, pp 177- 227.

(8) Loftsson T. and Brewster M.E. Pharmaceutical Applications of Cyclodextrins. 1. Drug Solubilization and Stablization. *J. Pharm. Sci.* 1996; 85 (10): 1017-1024.

(9) Loftsson T., Jarho P., Másson M. and Järvinen T. Cyclodextrins in drug delivery. *Drug Deliv.* 2005; 2 (2): 335-351.

(10) Szente L. and Szejtli J. Highly soluble cyclodextrin derivatives: chemistry, properties and trends in development. *Drug Deliv. Rev.* 1999: 36: 17-28.

(11) Higuchi T. and Connors K.A. Phase solubility techniques. *In Advances in Analytical Chemistry and Instrumentation.* Reilly, C.N. (Ed.); Interscience: New York, 1965; 4, pp 117-212.

(12) Aulton E.M. Pharmaceutics The Science of Dosage Form Design; Churchill Livingstone: New York. 2002, p 15-33.

(13) The European Pharmacopoeia, 7th ed., Vol. 2. Council of Europe, Strasbourg, France, 2011; p 2381.

(14) Barrett J., Smyth W.F. and Davidson I.E., An examination of acid-base equilibria of 1,4-benzodiazepines by spectrophotometry. *J. Pharm.* 1973; 25 (5): 387-393.

(15) Remington: The Science and Practice of Pharmacy;

Troy B.D. (Ed.); Lippincott Williams & Wilkins: Philadelphia, 2005; 21st edition, pp 222.

(16) Seedher N. and Bhatia S. Solubility enhancement of cox-2 inhibitors using various solvents systems. *AAPS Pharm. Sci. Tech.* 2003; 4 (3): 36-44.

(17) Viernstein H., Greiler-Weiss P. and Wolschann P. Solubility Enhancement of Low Soluble Biologically Active Compound Temperature and Cosolvent Dependent Inclusion Complexation. *Inter. J. Pharm.* 2003; 256 (1-2): 85-94.

(18) Rangel-Yagui C.O., Junior A.P. and Tavares L.C. Micellar Solubilization of Drugs. *J. Pharm. Sci.* 2005; 8 (2): 147-163.

(19) Alkhamis K.A., Allaboun H. and Al-Momani W.Y. Study of the Solubilization of Gliclazide by Aqueous Micellar Solutions. *J. Pharm. Sci.* 2003; 92 (4): 839-846.

(20) Loftsson T., Ma´sson M. and Brewster E.M. Self-association of cyclodextrins and cyclodextrin complexes. *J. Pharm. Sci.* 2004; 93: 1091-1099.

(21) Mosher G. and Thompson D.O. In: Encyclopedia of Pharmaceutical Technology. Swarbrick J. (Ed); Informa Healthcare USA, 2007; 3th edition, pp 671-696.

تطوير قابلية لورازبام لورازيبام للذوبان والإذابة

ياسمينة هاجيأبديتش[1] وأليسا إليزفتش[1] وبالما إلزوفتش[2] وإرفينا بابتش[2]

[1] كلية الصيدلة ، جامعة سراييفو، Čekaluša 90، 71000 سراييفو ، البوسنة والهرسك
[2] إدارة المنتجات الصيدلانية للتكنولوجيا، إدارة مراقبة المخدرات.

ملخص

لورازيبـــــام كمشتق البنزوديازيبينات ماده غير قابله للذوبان ويصعب التوصل لتركيز مناسب بمحلول مائي كالدواء السائل وأساليب تحسين قابلية ذوبانه في الماء: التخزين المؤقت وتخفيف التطبيق السطحي فالمركب حقق مدى ذوبان لورازباما تحت تاثير تغيير البيئه أو الرقم الهيدروجيني للمذيبات الثانويه كسطح المواد الفعاله والطرق المعقده وسيله لتحسين قابلية الذوبان استخدم (الايثانول والجلايكول البروبيلين والبولي ايثيلين جلايكول 200 إلي 400) مواد سطحيه فعاله صوربيتان البولي أوكسي monooleate (توين 80) complexation. Cosolvents (الإيثانول بروبيلين غليكول polyethyleneglyco l200 و400) السطحي البولي أوكسي monolaurate صوربيتان (توين20) والبولي أوكسي الأثير لوريل (بريج35) شولي الصوديوم وdeoxycholate الصوديوم وتوروكولات و[α−cyclodextrins cyclodextrin (α المضغوط)β cyclodextrin (β المضغوط) و2β هيدروكسي بروبيل 2) cyclodextrin إتش بي βCD) استخدمت كعوامل solubilizing في هذه الدراسة.
الهدف الرئيس هو ايجاد طريقه مناسبه تتيح ذوباناً جيداً واستخراجه مادة سائله. يمكن استنتاج أن التغيرات بقيم الرقم الهيدروجيني لا تؤدي إلى زيادة ذوبان ورازيبام وتحقق أفضل ذوبان في الماء لورازيبام باستخدام ماده سطحيه نشطه cyclodextrins.

الكلمات الدالة: لورازبام لورازيبام، ذوبان، الإذابة.

تاريخ استلام البحث 2011/3/9 وتاريخ قبوله للنشر 2012/1/2.