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Inclusion Complexation of Lorazepam with Different Cyclodextrins Suitable for Parenteral Use

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ABSTRACT The development of a parenteral lorazepam formulation, using cyclodextrins (CDs) as inclusion complexation agents, was investigated. CDs suitable for parenteral injection, i.e., hydroxypropyl-β-cyclodextrin (HP-β-CD), hydroxypropyl-γ-cyclodextrin (HP-γ-CD), sulfobutylether-7-β-cyclodextrin (SBE-7-β-CD), and maltosyl-β-cyclodextrin (malt-β-CD) were studied for the possibility to increase the solubility of lorazepam. Lorazepam interacted with all tested CD derivatives and 1:1 complexes are formed. HP-β-CD exerts the highest solubility improvement, reaching about 6 mg/ml lorazepam in 30% (w/v) CD solution. When using SBE-7-β-CD or malt-β-CD only half of that concentration can be dissolved. HP-γ-CD interacts much less with lorazepam. Parenteral solutions with 4 mg/ml in 30% (w/v) HP-β-CD solution, with 2 mg/ml in 30% (w/v) SBE-7-β-CD, and with 2 mg/ml lorazepam in 15% (w/v) HP-β-CD, were prepared. Sterile filtration of the formulation needs to be applied because of massive degradation of lorazepam during autoclaving. No precipitation is observed after dilution of the different formulations with (physiological) water or with 5% dextrose in water, which proves their suitability for administration with perfusions. The stability of the preparations was investigated in aqueous medium. During the first month, in all solutions more than 90% of lorazepam remained; after 3 months, less than 60% of lorazepam remained in the solutions with 15% (w/v) HP-β-CD and around 65–70% in the solutions with 30% (w/v) of CDs. Because of this short stability time, the preparations need to be lyophilized.

KEYWORDS Lorazepam, Cyclodextrins, Parenteral use, Inclusion complexation, Phase solubility, Stability

INTRODUCTION

Lorazepam or 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-2H-1,4-diazodiazepin-2-one belongs to the 1,4 benzodiazepine drugs. Benzodiazepines are widely used as minor tranquillizers, anticonvulsants, sedatives, muscle relaxants, and sleep inducers (KNMP, 1999; Martindale, 1999). The

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currently marketed formulations of lorazepam for parenteral use contain 4 mg/ml in the case of Temesta® (Wyeth Lederle) and 2 mg/ml or 4 mg/ml for Activan® (Wyeth) and lorazepam injection (Kanetta).

Lorazepam is practically insoluble in water (solubility: 0.08 mg/ml) (DAB, 1999; European Pharmacopoeia, 2002; The Merck-Index, 1996). Therefore, commercial injectable formulations of lorazepam usually are administered as solutions in polyols, such as polyethylene glycol 400 and propylene glycol (AHFS, American Hospital Formulary Service, 2002; Martindale, 1999; Trissel & Pearson, 1994). However, it is well-known that such formulations can cause serious side effects, such as pain and tissue damage at the site of injection [Brazeau & Fung, 1990; http://www.cyclodex.com/indexa.htlm (accessed May 2004); Medina et al., 2001]. Parenteral administration of the organic co-solvents also can cause hemolysis (Brazeau & Fung, 1989). Dilution of such formulations in aqueous media tend to precipitate (Li et al., 1998; Martindale, 1999) when injected, with consequences such as pain and phlebitis [Brazeau & Fung, 1990; http://www.cyclodex.com/indexa.htlm (accessed May 2004]).

An aqueous formulation would be more desirable, but then solubility problems occur. Therefore, an aqueous parenteral solution was developed with the aid of cyclodextrins (CDs) as complexing agents. The unique cyclic structure of CDs enables the formation of host-guest complexes by accommodating a wide variety of drug molecules inside their hydrophobic cavity. CDs have recently been used in pharmaceutical formulations of various drugs for the enhancement of solubility and/or stability (Holvoet et al., 2003; Loftsson & Brewster, 1996, 1997; Narisawa & Stella, 1998). The use of native β-CD is limited, due to its low solubility and significant renal toxicity after parenteral administration. Many β-CD derivatives with an improved aqueous solubility and a reduced acute systemic toxicity profile have been synthetized (Irie & Uekama, 1997; Ma et al., 2000). The native γ-CD and the hydrophylic β-CD derivatives (for instance, HP-β-CD) can be used in parenteral dosage forms due to their intravenous safety (Irie & Uekama, 1997; Loftsson & Brewster, 1997; Ma et al., 2000). Hydroxyalkylated CD derivatives (HP-β-CD and HP-γ-CD) have been proved very useful in intravenous and other parenteral preparations because of their low hemolytic activity and irritation compared to β-CD and its alkylated forms. HP-β-CD is the most accepted representative of the hydroxyalkylated derivatives to use as a hydrophilic drug carrier because of its amorphousness, high water solubility and solubilizing power, low cost, and toxicity (Archontaki et al., 2002). Because malt-β-CD is considered to be safe in most circumstances (Loftsson & Brewster, 1997), it is also examined in this study. The other tested CD was SBE-7-β-CD because it seems suitable in parenteral formulations (Irie & Uekama, 1997; Loftsson & Brewster, 1997).

The aim of this work was to make a comparative study of the solubility enhancement of lorazepam caused by its complexation with different CDs. These results were used to prepare and evaluate parenteral formulations.

MATERIALS AND METHODS

Chemicals

Lorazepam (MW=321.2) was obtained from Alpha Pharma (Zwevegem, Belgium), hydroxypropyl-β-cyclodextrin (HP-β-CD, DS=4.2, MW=1380) from Roquette (Lestrem, France), and sulfobutylether-7-β-cyclodextrin (SBE-7-β-CD, DS=6.7, MW=2200) (Captisol®) from Cydex (Overland Park, Kansas). The other tested CD derivatives hydroxypropyl-γ-cyclodextrin (HP-γ-CD, DS=6.9, MW=1703) and maltosyl-β-cyclodextrin (malt-β-CD, DS=2.8, MW=1796) were purchased from Cyclodextrin Technology Development (High Springs, Florida). Methanol (MeOH) HyperSolv® for HPLC (BDH Laboratory Supplies, Poole, England) was used. Hydrochloric acid (HCl) 37% and sodium hydroxide (NaOH) pellets were obtained from Merck (Darmstadt, Germany) and hydrogen peroxide from Federa (Brussels, Belgium). For filtration, Teflon or PVDF (polyvinylidene difluoride) membrane filters with a pore size of 0.2 μm (Macherey Nagel, Düren, Germany) were used. Sodium chloride (NaCl) and dextrose were purchased from Merck and UCB (Brussels, Belgium), respectively.

Apparatus

The equipment used for the high performance liquid chromatography (HPLC) assay included a L-6000

pump (Merck-Hitachi, Tokio, Japan) equipped with a Rheodyne 7125-075 injector (Cotati, California) containing a 20 µl loop, and a variable wavelength Perkin Elmer LC 90 UV spectrophotometric detector (Shelton, Connecticut). The HPLC assay of lorazepam was conducted with a reversed phase (RP) C_{18}, Lichrospher® 100, 250 × 4 mm; 5 µm column in connection with a C_{18} guard column Lichrospher® 100, 4 × 4 mm; 5 µm, both from Merck.

In all experiments, mQ water obtained from a mQ water purification system was used (Millipore, Molsheim, France). pH measurements were performed using a Radiometer Copenhagen PHM 26 pH meter (Copenhagen, Denmark) calibrated daily using pH 4.00, 7.00, and 10.00 standard buffers (Merck). The pH of small volumes was measured with a WTW Multical pH meter (Metro Parkway, Florida). Isotonicity was measured with a Knauer osmometer (Berlin, Germany). During forced degradation, an oven (Memmert, Schwabach, Germany) adjusted to 45°C and a Universal UV-lamp of 366 nm (Camag, Muttenz, Switzerland) were used.

High-Performance Liquid Chromatography Analysis

The HPLC method consisted of an isocratic elution with a MeOH-water mobile phase (60/40, v/v). The flow rate was 1.0 ml/min and the detection wavelength 316 nm. Analyses were performed at ambient room temperature (approximately 20°C).

Standard Preparation

A stock solution of 1.0 mg/ml lorazepam in mobile phase was prepared by ultrasonification during 1 min. The standard solution was obtained by diluting the stock solution 5 times with the mobile phase.

Linearity

Calibration curves were prepared using 10 standards, containing 0.01, 0.02, 0.05, 0.11, 0.15, 0.21, 0.25, 0.31, 0.42, and 0.50 mg lorazepam/ml mobile phase, by injecting three times each standard.

Precision

The standard containing 0.21 mg/ml was six times prepared and injected. The repeatability of injection was established by making six replicate injections of two different lorazepam standard concentrations (0.1 and 0.2 mg/ml) and of four lorazepam-cyclodextrin solutions with ca. 1 mg/ml lorazepam.

Accuracy

The selected formulations of lorazepam were prepared: 4 mg lorazepam was dissolved in 1 ml 30% (w/v) HP-β-CD or in 2 ml 30% (w/v) SBE-7-β-CD and 2 mg lorazepam was dissolved in 1 ml 15% (w/v) HP-β-CD. Each formulation was prepared and injected 3 times on the HPLC system after a 10 and 20 times dilution of the initial concentrations of 2 mg/ml and 4 mg/ml, respectively.

Degradation of Lorazepam

Degradation of lorazepam under extreme basic or acidic conditions: circa 50 mg lorazepam was stored at 45°C in 25 ml of 2N NaOH or of 2N HCl and protected from light. Before injection, the supernatant of the samples was diluted 10 times with mobile phase. An acidic and basic blank were also injected.

Degradation of lorazepam in hydrogen peroxide: a suspension of circa 50 mg lorazepam was prepared in 25 ml of 3% of hydrogen peroxide, and stored at 25°C. The five times diluted supernatant was injected after 1 day.

Degradation of lorazepam under influence of light: 100 mg lorazepam in 100 ml mobile phase was irradiated with an UV lamp of 366 nm during 2 days at ambient temperature. Before injecting, the sample was diluted five times with mobile phase.

Solubility Studies

A range of different concentrations of CD [1, 2, 5, 10, 15, 20, 25, and 30% (w/v)] in water (non-adjusted and adjusted to pH 5.0 by adding 0.1 N HCl) was prepared. An excess of lorazepam was added to 5.0 ml of each CD solution in screw-capped test tubes. These preparations were shaken during 5 days, up to equilibrium, in a thermostatically controlled water bath shaker at 25°C. To avoid evaporation, the tube cups were wrapped in parafilm. After equilibrium, the suspensions were centrifugated at 1787 g for 20 min. The supernatant was carefully removed. To elimate remaining particles, the supernatant was filtered

through a Teflon or PVDF filter. The filtrate contained both dissolved lorazepam forms: complexed and non-complexed. The filtrate was diluted with mobile phase, prior to injection in the HPLC system, to ensure that the lorazepam concentration was situated in the calibration range (see "Evaluation of the HPLC Method", p. XXX). Each sample was injected three times and the peak areas were averaged.

Preparation and Evaluation of the Parenteral Formulations

The results from phase solubility studies were used to prepare parenteral formulations with two parenterally safe cyclodextrins. The parenteral formulation of lorazepam with HP-β-CD was prepared as follows: 40 mg lorazepam was equilibrated with 10 ml of 30% (w/v) HP-β-CD in water. The mixture was vortexed for about 10 min and then stirred at room temperature until all lorazepam was dissolved (approximately 3 h). The solution was sterilized through a 0.2 μm membrane filter and 1 ml of the solution was aseptically transmitted in a sterile vial, which was closed immediately. The preparation was frozen during 15 min at $-45°C$ and the formulations were lyophilized during 24 h. Before use, the lyophilised product has to be redissolved in 1 ml sterile water.

Other formulations containing 4 mg lorazepam in 2 ml of 30% (w/v) SBE-7-β-CD and 2 mg lorazepam in 1 ml of 15% (w/v) HP-β-CD in water were prepared similarly.

Clarity

The formulations were sequentially diluted in one-to-one ratio (Li et al., 1998) with the following diluents: water, 0.9% NaCl in water, 5% dextrose in water. To the selected formulation, an equal volume of dilution solution is added. After vortexing, each solution is visually evaluated for precipitation (both with a magnifying glass against a black and white background, and under the microscope). Subsequently, a fraction of this dilution is further diluted and checked for precipitation. This process is repeated five times.

Stability Study

The samples, i.e., the three above mentioned parenteral formulations, were stored in a thermostatically controlled room of 25°C during stability studies. The samples containing 2 mg/ml were diluted 10 times, those containing 4 mg/ml were diluted 20 times, before injection in the HPLC system. The concentration in the sample was estimated relatively to the 0.20 mg/ml lorazepam standard by means of a one-point calibration.

RESULTS AND DISCUSSION

Evaluation of the HPLC Method

The HPLC method was selected based on the work of Carpenter et al. (1981). The method was validated in term of linearity between 0.01–0.5 mg/ml ($r=0.9999$). The repeatability of the standard was less than 1.15%. The repeatability of the injection was investigated by performing six determinations of lorazepam and of lorazepam-CD samples in which the relative standard deviation (RSD) was $\leq 0.76\%$. Therefore the method is considered precise (European Pharmacopoeia, 2002).

Before studying the solubility enhancement of lorazepam caused by its complexation with HP-β-CD, HP-γ-CD, SBE-7-β-CD, or malt-β-CD, the interference of 4% of the different CDs in the chromatogram was checked. This CD concentration is the highest one occurring in the samples for analysis. Neither HP-β-CD, HP-γ-CD, SBE-7-β-CD, nor malt-β-CD interfered with the lorazepam peak. Only a solvent peak was noticed, due to the UV transparency of the CDs.

The solutions of lorazepam were prepared at the 100% level of the selected formulations (see further) and chromatographed versus a lorazepam standard (Fig. 1). The data show that quantitative recovery of lorazepam is obtained (values between 99.0% and 100.8%) and that the method of analysis is accurate.

The HPLC-method was also checked on its ability to investigate results from stability studies. Possible degradation products were created by submitting lorazepam to extreme conditions. After degradation of lorazepam under extreme basic conditions (1 h), the small remaining lorazepam peak is separated from the degradation products (Fig. 2a). Following degradation of lorazepam under extreme acidic conditions, the lorazepam peak was separated from its degradation products in the above defined HPLC system when the sample was injected after 1 h (Fig. 2b). After

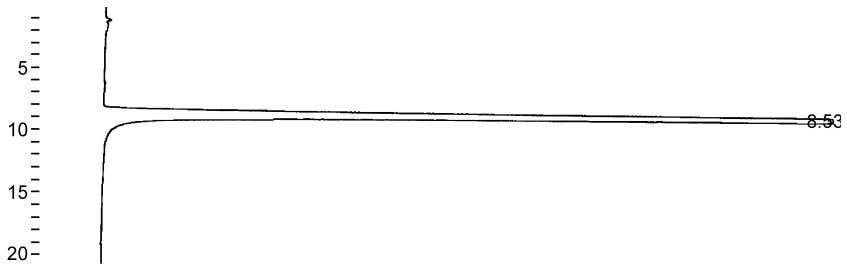

FIGURE 1 **Chromatogram of 0.2 mg/ml Lorazepam. Conditions: Mobile Phase 60/40 (v/v) MeOH-Water, Stationary Phase RP-C_{18}, Flow-Rate 1 ml/min, Detection Wavelength 316 nm.**

degradation testing of lorazepam in hydrogen peroxide and under the influence of light, for the executed conditions, no degradation products were observed.

The described HPLC system [MeOH-water: 60/40 (v/v)] was thus found suitable for stability-indicating measurements until a degradation of approximately 50% of lorazepam, which is far beyond the degradation limits considered in this study.

Solubility Studies

To determine the best combination of a CD and lorazepam, i.e., the one improving the solubility most, it is necessary to measure the solubility curves of lorazepam in aqueous CD solutions. These curves then can be used as a basis for preparing a parenteral solution, containing the required amounts of 2 or 4 mg lorazepam. The concentration of CD needed to incorporate the desired amount of lorazepam is, of course, an important factor to choose a given CD derivative, but also the cost of the CD itself and its contribution to osmolality needs some attention, e.g., a given amount of SBE-7-β-CD contributes more to tonicity than HP-β-CD.

The phase solubility diagrams were drawn by plotting the experimentally determined concentrations of lorazepam (mg/ml) ($n=2$, RSD $\leq 2.5\%$) as a function of the % (w/v) CD concentration for all replicates. This representation gives direct information about the complexation efficiency. The complexation

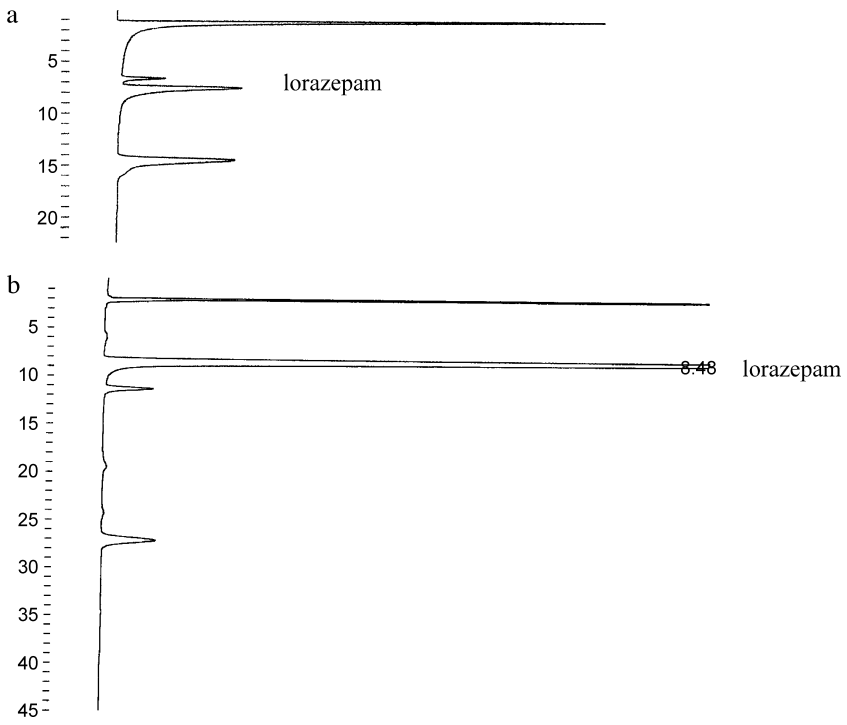

FIGURE 2 **Chromatogram of Lorazepam Stored 1 h Under Basic (2a) and Acidic (2b) Conditions. Conditions: Mobile Phase 60/40 (v/v) MeOH-Water, Stationary Phase RP-C_{18}, Flow-Rate 1 ml/min, Detection Wavelength 316 nm.**

of lorazepam in the different CDs at 25°C can be seen in Fig. 3. Since similar results were obtained, the solubility curves performed at pH 5.0, were not shown. It could be foreseen that there was no difference in the results between the solubility curves in water: adjusted and non-adjusted (pH 5.7) to pH 5.0 by adding 0.1 N HCl, as the pKa values of lorazepam are 1.3 and 11.5 (AHFS, 2002) and the cyclodextrins used are neutral, excepted for SBE-7-β-CD.

Lorazepam interacted with the four CD derivatives; the complexed amount of lorazepam increases linearly with the CD concentration. HP-β-CD has the highest complexation efficiency. At a given CD concentration, SBE-7-β-CD or malt-β-CD dissolve only about half of the amount of lorazepam, compared to HP-β-CD. The incorporation in HP-γ-CD is low compared to the other CD derivatives.

The solubility of lorazepam in water is reported to be 0.08 mg/ml (DAB, 1999; European Pharmacopoeia, 2002; The Merck-Index, 1996), i.e., 50 times below the targeted concentration of 4 mg/ml, occurring in the currently marketed formulations. The solubility of lorazepam, found from our own measurements, was 0.063 mg/ml at 25°C (n=8, RSD=1.3%) and 0.055 mg/ml at 4°C (n=8, RSD=1.0%). (Newton et al. 1983) found a solubility of 0.054 mg/ml in deionized water. The solubility enhancement of lorazepam in 10% HP-β-CD is reported to be 32 times compared to lorazepam's own solubility (Roquette, 2002). Our results for HP-β-CD were similar: an enhancement of 27 times at 25°C and of 33 times at 4°C was found.

To determine the type of complexation between lorazepam and CD, the concentrations of lorazepam in mol/l are plotted as a function of the molar CD concentration (figure not shown since similar to Fig. 3). In the CD concentration range studied, the solubility diagrams can be classified as an A_L type, describing a linear increase in solubility of activum as a function of CD (Higuchi & Connors, 1965). Because such linear profiles of the phase solubility diagrams are characterized by a slope below one, it is assumed that the solubility increase is due to the formation of a 1:1 complex (Higuchi & Connors, 1965). The formation constants of such complexes, $K_{1:1}$ or K_c, are calculated by the equation:

$$K_c = \text{slope}/[S_0(1 - \text{slope})] \qquad (1)$$

where S_0 is the intrinsic solubility of the drug in water. The stability constants varied from 82 M^{-1} up to 399 M^{-1} at 25°C for HP-γ-CD and HP-β-CD, respectively. The K_c value for SBE-7-β-CD was 323 M^{-1} and for malt-β-CD it was 258 M^{-1}. The magnitude of the binding constant of CD-drug complexes has been reported in the range of $0-10^5$ M^{-1}, with 0 M^{-1} being the value for a drug incapable of forming an inclusion complex (Rajewski & Stella, 1996). The stability constant of HP-β-CD is higher than that of HP-γ-CD. The same observation was reported by (Trapani et al. 2000) for the complexation of zolpidem with HP-β-CD and HP-γ-CD. By comparing different K_c values for complexation between a benzodiazepine and a CD, those found for lorazepam seem relatively high.

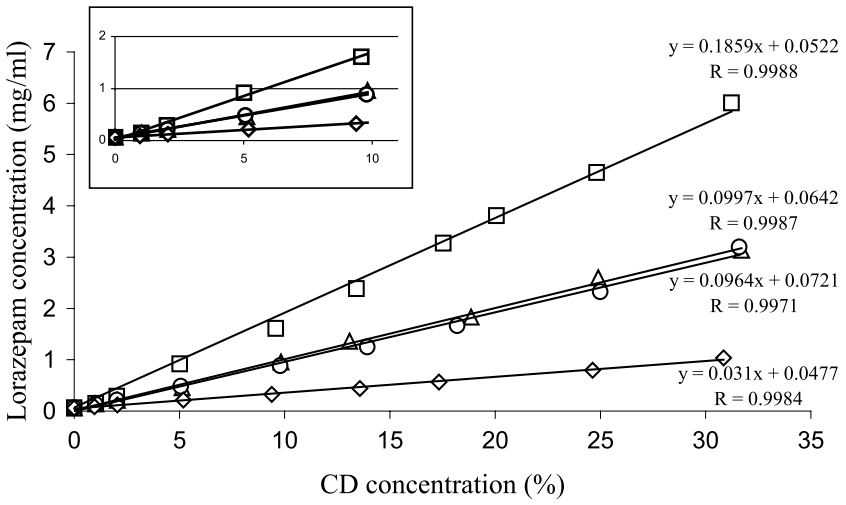

FIGURE 3 Concentration of Lorazepam (mg/ml) as a Function of the CD Concentration [%(w/v)] at 25°C. Key: □ HP-β-CD, △ SBE-7-β-CD, ○ Malt-β-CD, ◇ HP-γ-CD.

(Yamamoto et al. 1989), for instance, found a K_c of 220 M^{-1} for diazepam with malt-β-CD. We found comparable values for lorazepam. Stability constants of only 70–75 M^{-1} were found for the complexation between bromazepam and HP-β-CD (Archontaki et al., 2002). The higher the K_c value, the more lorazepam is bound and the slower its release (Holvoet et al., 2003). For preparing a formulation for intravenous administration, the magnitude of the affinity constant is important that with K_c values higher than 10,000 M^{-1} the dilution factor may be insufficient for a complete release of the drug. However, with the obtained K_c values, which are a few hundred, the release of lorazepam cannot pose problems.

Preparation and Evaluation of the Parenteral Formulations

The currently marketed parenteral formulations contain 2 or 4 mg lorazepam per ml polyethylene glycol 400, propylene glycol, and benzyl alcohol 2% (AHFS, 2002). The solubility of lorazepam in water is notably affected by the presence of CD (Fig. 3). Up to almost 6 mg/ml can be dissolved in 30% (w/v) of HP-β-CD and approximately half of it using malt-β-CD or SBE-7-β-CD. Thus, a 30% (w/v) HP-β-CD solution allows a lorazepam concentration corresponding to about an 100-fold increase in solubility compared to its intrinsic solubility in an aqueous system.

The required 4 mg lorazepam can, according to Fig. 3, be prepared in 1 ml of a 22% (w/v) HP-β-CD solution. However, in practice this amount was still not completely dissolved after 1 day. The reason was that the CD concentration was too close to the saturated concentration and therefore we decided to increase it to 30% in order to shorten the preparation time and to ensure that the preparation will not precipitate due to temperature changes, e.g., stored at 4°C.

In summary, the targeted amount of 4 mg lorazepam is achieved when preparing complexes of lorazepam in 1 ml of a 30% (w/v) HP-β-CD solution or in 2 ml of a 30% (w/v) SBE-7-β-CD solution. Also, preparations of 2 mg lorazepam in 1 ml of a 15% (w/v) HP-β-CD and of 2 mg lorazepam in 1 ml of a 30% (w/v) SBE-7-β-CD were made. Suitable preparations with malt-β-CD also are possible, but due to unavailability of the substance (only available on the Japanese market), preparations with this CD derivative were not further examined.

Quality requirements for parenteralia such as control of pH, isotonicity, sterility, clarity, compatibility with the packing material, and stability during the storage process were evaluated.

pH

The pH of the different prepared formulations was around 5.7, which is conforming because above pH 9, tissue necrosis may occur and below pH 3, extreme pain and phlebitis may occur (DeLuca & Boylan, 1992).

Isotonicity

The solution of 2 mg lorazepam in 1 ml 15% (w/v) HP-β-CD in water is hypotonic (120 mosmol/kg) and was made isotonic by adding NaCl. The lorazepam solution with HP-β-CD is considered more suitable for parenteral use since it is nearly iso-osmotic while the formulation with SBE-7-β-CD is hypertonic.

Sterility

Sterilization by autoclaving is not feasible as lorazepam degrades extremely rapidly [14% recovery ($n=2$, RSD=1.6%, 1 formulation)]. Sterile filtration can be applied [99.4% recovery ($n=1$, each formulation)].

Clarity

It is described that 1:1 complexes do not precipitate by diluting them (Rajewski & Stella, 1996), a statement that was experimentally verified by the static serial dilution method (Li et al., 1998). Lorazepam did not precipitate in any of the respective media [(physiological) water and water with 5% dextrose], which indicates the suitability of the parenteral formulations for administration with perfusions.

Compatibility with Packing Material

Lorazepam should not be added to or stored in polyvinyl chloride (PVC) bags containing 0.9% sodium chloride or 5% dextrose perfusion solutions due to adsorption (Higuchi & Connors, 1965; Hoey et al., 1994; Mc Guire et al., 1993; Trissel & Pearson, 1994). However, other authors did not find lorazepam

adsorbed either to PVC bags (Martens et al., 1990) or to various intravenous tubings (Carpenter et al., 1981). To avoid all risks, our preparations were stored in small glass bottles during the stability study.

Stability Study

The chemical stability of the aqueous selected lorazepam preparations was evaluated. The pH changed during the stability study (Table 1). It decreased in the formulations with HP-β-CD. The pH of the lorazepam formulation with SBE-7-β-CD remained about constant. The reason herefore remains unknown to us, but at first sight seems to be related to the CD used. Even the selected preparations with initial pH 5.0 could not retain their pH. pH 5.0 was also selected as it is the pH of maximum lorazepam stability (DAB, 1999).

The preparations with 30% (w/v) CD seem to be the most stable, while those with only 15% (w/v) HP-β-CD were the least (Fig. 4). In general, it can be concluded that the stability curves of the formulations with SBE-7-β-CD or HP-β-CD are relatively similar. The stability of lorazepam in preparations with pH adjustment to pH 5.0 was similar (figure not shown since similar to Fig. 4). An explanation might be due to the fact that both pH values were not very different.

During the first month, in all solutions more than 90% of lorazepam remained. After 3 months less than 60% of lorazepam remained in the solutions with 15% (w/v) HP-β-CD, and around 65–70% in the solutions with 30% (w/v) of CDs.

For most injectable products, either compendia or governmental regulations require that a formulation does not contain less than 90% of the label claim of active ingredient at the expiration date (DeLuca & Boylan, 1992). The shelf life of the different formulations examined is only one month, which is insufficient to be of industrial interest.

TABLE 1 **pH Change During the Stability Study of the Lorazepam Formulations**

Lorazepam formulation	pH at day 0	pH after 100 days, at the end of the experiment
2 mg in 1 ml of 15% HP-β-CD	5.78	3.8
4 mg in 1 ml of 30% HP-β-CD	5.73	3.3
4 mg in 2 ml of 30% SBE-7-β-CD	5.76	5.6

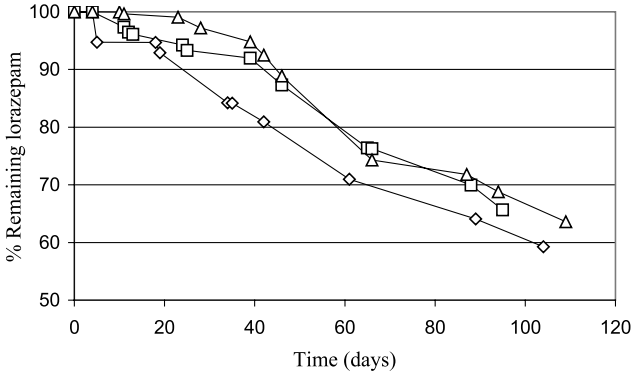

FIGURE 4 **Stability Curves of the Different Lorazepam Formulations. Key: ◇ 2 mg Lorazepam in 1 ml of 15% HP-β-CD, □ 4 mg Lorazepam in 1 ml of 30% HP-β-CD, △ 2 mg Lorazepam in 1 ml of 30% SBE-7-β-CD.**

To overcome such problems, formulations can be lyophilized, which consequently was done. The lyophilized preparations were clear after reconstitution with water, while lyophilization and reconstruction did not change pH nor isotonicity, indicating that the lyophilization will not introduce technical problems for industrial scale. The lyophilisates were stable during the tested period of 1.5 months. However, the long-term stability of these lyophilisates, prepared on industrial scale, remains to be studied.

CONCLUSIONS

The commercial formulations of lorazepam for parenteral use are solutions in organic solvents having a number of side effects, such as pain, tissue damage at site of injection, hemolysis, and phlebitis, when applied. This study evaluated the effects of various CD derivatives (HP-β-CD, HP-γ-CD, SBE-7-β-CD, and malt-β-CD) on the solubilization of lorazepam. The target doses of 2 or 4 mg lorazepam were achieved by preparing inclusion complexes of 4 mg lorazepam in 1 ml 30% (w/v) HP-β-CD or in 2 ml 30% (w/v) SBE-7-β-CD, and of 2 mg lorazepam in 1 ml of 15% (w/v) HP-β-CD or 1 ml of 30% (w/v) SBE-7-β-CD. The preparations fulfilled the requirements for parenteral formulations. They can be used in parenteral injections and in perfusions. The stability of the preparations is evaluated and the solutions remained stable only during a period of one month. Therefore, the preparations were lyophilized as this might

improve their stability. On lab scale, a stability during the tested period of 1.5 months was obtained.

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