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Development of a non-surfactant parenteral formulation of miconazole by the use of cyclodextrins

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Abstract

Miconazole is an antimycotic drug exhibiting a very poor water solubility (< 1.03 μg/ml). It has been shown that cyclodextrins (CDs) are able to form inclusion complexes with miconazole and that they are able to increase its aqueous solubility. Miconazole is a weak base whose solubility depends of the pH. The purpose of this study was to investigate the influence of both CDs and different acids on the solubility of miconazole. It was found that a synergistic effect existed between CDs and different acids. The combination of hydroxypropyl-βCD (HP-βCD) (100 mM) or sulfobutylether 7-βCD (SBE_7-βCD) (50 mM) and lactic acid (50 mM) allowed to dissolve more than 10 mg of miconazole per ml. NMR studies confirmed the formation of an inclusion complex miconazole–CD in an acidic medium. It was also shown by the NMR studies that the complex formed was a 1:1 complex. These results demonstrate that it is possible to develop a parenteral aqueous solution of miconazole without surfactant.

Keywords: Cyclodextrins; Miconazole; Parenteral solution; Polyoxyl 35 castor oil

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of covalently linked glucopyranose rings. α-, β- and γCD are naturally occurring CDs combining six, seven or eight glucopyranose units. The CDs and their derivatives are used in pharmaceutical formulations to enhance solubility, dissolution rate, stability and bioavailability (Frömming and Szejtli, 1994).

CDs are able to form inclusion complexes with many lipophilic drugs, thus changing the physico-

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chemical and biopharmaceutical properties of the drugs.

Antimycotic imidazole derivatives are lipophilic drugs, and complexation of these drugs with CDs has been studied by several authors (Van Doorne et al., 1988a,b; Mura et al., 1992; Pedersen et al., 1993a,b; Pedersen, 1994; Bononi, 1995).

Miconazole (1-(2-((2,4-dichlorophenyl)-2-(2,4-dichlorophenyl)-methoxy)ethyl)-1-imidazole) is a compound well known for its antimycotic activity (Fig. 1). More particularly, miconazole is an antimycotic drug with a wide activity spectrum; it is endowed with a powerful activity against dermatophytes and *Candida albicans*, as well as against some Gram-positive germs.

Unfortunately, miconazole is practically insoluble in water (< 1.03 μg/ml) and is consequently formulated with a non-ionic surfactant, Cremophor® (polyoxyl 35 castor oil), for parenteral administration. Polyoxyl 35 castor oil is associated with several side effects most notably an allergic reaction comparable with anaphylactic shock (Howrie et al., 1984; Hopkins, 1988; Brewster et al., 1989; Reynolds and Aronson, 1992). An aqueous formulation of miconazole which does not contain this surfactant may, therefore, be useful.

Miconazole is a weak base ($pK_a = 6.7$) with a pH-dependent solubility.

In this work, the combined effect of water-soluble cyclodextrins and acids is studied in order to develop an alternative parenteral formulation containing miconazole.

2. Materials and methods

2.1. Materials

Miconazole was obtained from Janssen Pharmaceutica (Beerse, Belgium). βCD, γCD and hydroxypropyl-βCD (HP-βCD) were obtained from CNI (Neuilly-sur-Seine, France), Wacker Chemie GmbH (Munchen, Germany) and Janssen Biotech (Olen, Belgium), respectively. Sulfobutylether 7-βCD (SBE_7-βCD) was kindly supplied by Cydex (Kansas). All other products were of analytical grade.

2.2. Phase solubility studies

Solubility studies were performed as described by Higuchi and Connors (1965). Excess amounts of miconazole were added to various concentrations of SBE_7-βCD in 10 ml of purified water. The suspensions were shaken in a water bath at 25°C during 48 h. An aliquot was filtered through a 0.45-μm filter and assayed for miconazole content by HPLC.

2.3. Miconazole assay

Miconazole can be determined using an HPLC method. The HPLC system consisted of a L-6000 Merck–Hitachi high pressure pump connected to an L 4000 Merck–Hitachi UV detector set at 230 nm. Data were analyzed with a D 2500 Merck–Hitachi chromato-integrator.

Twenty-μl samples were injected on a Lichrocart column (125×4 mm i.d.) prepared with an octylsilane (C8) phase Lichrospher 60 RP-Select B 5 μm (Merck) and maintained at 27°C. The mobile phase consisted of a 70:30 (v:v) mixture of methanol HPLC grade and 0.05 M acetate buffer, pH 3.5; the flow rate was 1.0 ml/min. All the samples were analyzed in duplicate. Each sample was diluted with mobile phase before injection in the HPLC system. This method was validated (Caporal-Gauthier et al., 1992) and showed a good linearity, reproducibility and accuracy.

The limits of detection (LOD) and quantification (LOQ) were determined from the regression line equation and were found to be equal to 1.03 μg/ml for the LOD and 3.42 μg/ml for the LOQ.

2.4. Solubility studies

The aqueous solubility of miconazole was measured in different concentrations of different acids with or without addition of CDs: an excess amount of miconazole was added to 10 ml of solution containing a given concentration of acids and/or CD. After shaking in a water bath at 25°C for 24 h, the suspension was filtered through a 0.45-μm filter and the miconazole was assayed by HPLC.

2.5. ^1H NMR

^{1}H NMR measurements were performed with a Brucker DRX-400 Avance spectrometer operating at 400.13 MHz. In order to prove the inclusion in an acidic medium, samples were dissolved in a 0.1 M phosphate buffer solution (pD3) (H_3PO_4 0.1 M in D_2O; added NaOD up to pD3). Chemical shifts are given relative to external tetramethylsilane at 0 ppm.

The continuous variation method experiments were carried out under the same conditions. The sum of the two components was kept constant ([miconazole] + [CD] = 5 mM) but the molar fraction of each component (r = [miconazole]/[miconazole] + [CD] or r = [CD]/[CD] + [miconazole]) ranged between 0 and 1.

2.6. Stability studies

The stability of the parenteral solution was measured at 4, 25 and 45°C. Miconazole was assayed just after the preparation of the batch (T_0), after 1 and 2 weeks, and 1, 2, 3 and 6 months. Miconazole was assayed by HPLC as described before. The pH value was verified after 1, 3 and 6 months. The solution was also checked visually for the absence of precipitation.

3. Results and discussion

3.1. Effect of CDs on the aqueous solubility of miconazole

It was already shown in the literature that α-, β- and HP-βCD were able to form inclusion complexes with miconazole and that they were able to increase its aqueous solubility (Van Doorne et al., 1988a,b; Mura et al., 1992; Pedersen et al., 1993a,b; Pedersen, 1994; Bononi, 1995).

Fig. 2 shows that SBE$_7$-βCD is also able to increase the aqueous solubility of miconazole. The aqueous solubility of miconazole increases as a function of the concentration of CD and in the CD concentration range studied the solubility diagram can be classified as A_L. The apparent stability constant (K_c or $K_{1:1}$) was calculated from the following equation:

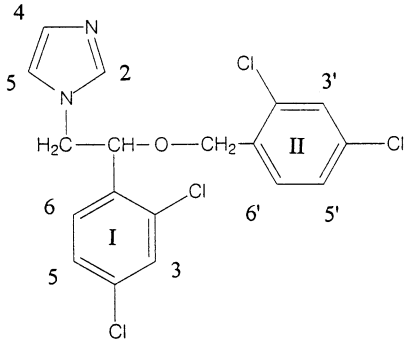

Fig. 1. Chemical structure of the miconazole.

$$K_c = \frac{s}{s_0 \cdot (1 - s)}$$

where s is the slope of the A_L diagram and S_0 is the solubility of miconazole without CDs. The apparent stability constant calculated ($K_{1:1}$) is around 3×10^6 M^{-1}. The high K_c value obtained suggests that, in purified water, the cavity of this CD derivative accommodates very well the molecular portion of miconazole involved in the inclusion. The intrinsic solubility of miconazole in water was less than 1.03 μg/ml. This value was the limit of detection of the HPLC method and was considered as the intrinsic solubility. At the same CD concentration (10 mM), the increase of the aqueous solubility of miconazole is 9, 29 and 68 times with HP-βCD, βCD and SBE$_7$-βCD, respectively.

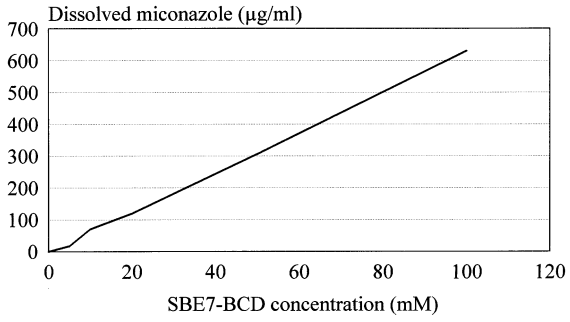

Fig. 2. Phase solubility diagram of the miconazole with SBE$_7$-βCD.

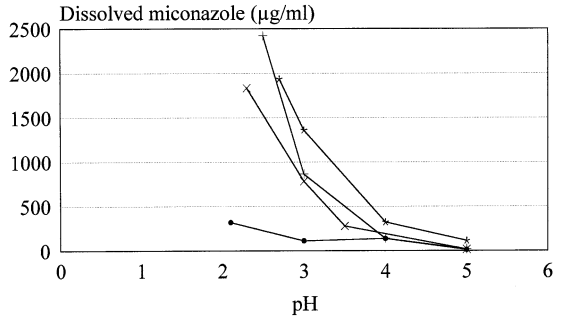

Fig. 3. Solubility of miconazole (μg/ml) in different 50 mM buffer solutions: citrate (●), lactate (+), gluconate (*) or phosphate (×) buffer solutions.

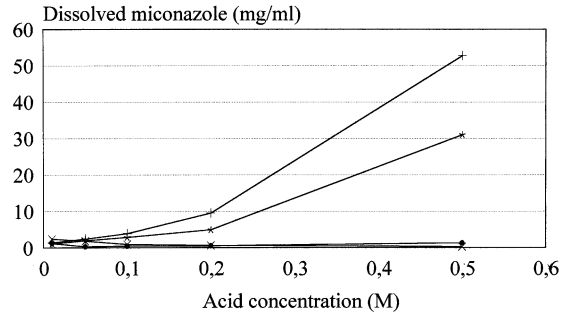

Fig. 4. Solubility of miconazole (mg/ml) in function of the concentration of citric (●), lactic (+), gluconic (*) or phosphoric (×) acid.

3.2. Effect of different acids on the aqueous solubility of miconazole

Miconazole is a weak base ($pK_a = 6.7$) whose solubility was studied in different acids.

Firstly, we studied the solubility of miconazole as a function of the pH. Buffer solutions (50 mM) were prepared with different acids (citric, lactic, gluconic and phosphoric) by addition of NaOH up to pH 3.0, 4.0 or 5.0. The solubility of miconazole was measured in these buffer solutions and in 50 mM solutions of the acid without addition of NaOH. The pH value of the solutions was controlled after the experiment. The results obtained with the four different buffer solutions are shown in Fig. 3. At pH 3, the solubility of miconazole is, respectively, equal to 200, 864, 1359 and 783 μg/ml in citrate, lactate, gluconate and phosphate buffer solutions. Lactate and gluconate buffers seem to be the most interesting ones.

Secondly, to confirm these differences, the solubility of miconazole was measured as a function of the concentration of the same acids. Fig. 4 confirms that lactic and gluconic acids have a more pronounced effect than citric and phosphoric acids on the solubility of miconazole. The observed solubility values are not due to differences of the pH value: the pH value of a 500 mM phosphoric acid solution is around 1.3, whereas the pH value of a 500 mM lactic or gluconic acid solution is around 2.7. The observed solubility values might be explained by differences of the solubility of the salts formed between miconazole and the acids. To confirm this hypothesis, we tried to isolate the corresponding salts. Miconazole and the corresponding salts in a 1:1 ratio were solubilized in alcohol and then evaporated up to precipitation. The lactic and gluconic miconazole salts did not precipitate probably because of their too high solubility, contrary to the phosphate miconazole salt, for example, which precipitates because of its low solubility. The nature of the acid is the most important factor.

3.3. Combined effect of acids and CDs on the aqueous solubility of miconazole

Phase solubility diagrams were performed in 50 mM acid solutions (Fig. 5). The diagrams can be classified as A_N type for all acids (this is in accordance with the results reported by Pedersen

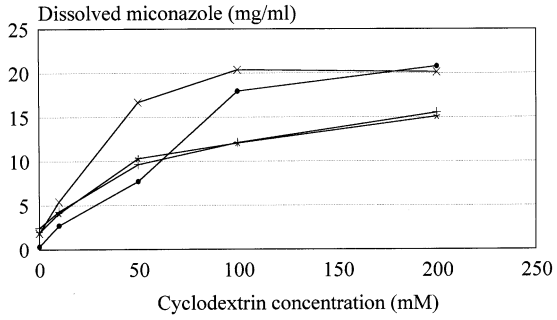

Fig. 5. Phase solubility diagram of miconazole with HP-βCD in 0.05 M citric (●), lactic (+), gluconic (*) or phosphoric (×) acid solutions.

Table 1
Solubility of miconazole (μg/ml) in water and in different acids (50 mM) in absence or in presence of HP-βCD or SBE$_7$-βCD (50 mM)

	Without CD	With HP-βCD 50 mM	With SBE$_7$-βCD 50 mM
Water	<1.03	76.00	305.80
Lactic acid	2420.00	9626.00	15 134.00
Gluconic acid	1935.00	10 292.00	15 300.00
Phosphoric acid	1833.00	16 251.00	22 481.00
Citric acid	321.00	7698.00	22 064.00

et al. (1993a,b). The same profiles were obtained with SBE$_7$-βCD. The apparent stability constants are much lower than that obtained in purified water. This is due to the fact that in 50 mM acid solutions, the measured pH values are between 3.2 and 4.1. At these pH values, miconazole is ionized and its affinity to the CD's cavity decreases. These diagrams show that the interaction between miconazole and CDs also exist in acidic medium.

The combined effects of acids and CDs on the solubility of miconazole were studied under optimal conditions to develop an injectable solution. A concentration in acid equal to 50 mM was chosen to avoid a too low pH value of the solution since intravenous solutions should have a pH value between 3 and 10.5. Among the different available CDs, HP-βCD and SBE$_7$-βCD were chosen owing to their high solubilizing power and their low systemic toxicity relative to βCD (Yoshida, 1988; Brewster et al., 1990; Rajewski et al., 1995; Stella, 1996).

Combined effects of acids and HP-βCD were already mentioned in a previous paper (Piel et al., 1997). The following results also report the effect of SBE$_7$-βCD. Table 1 gives the values of the solubility of miconazole without CD, with HP-βCD (50 mM) (Piel et al., 1997) and with SBE$_7$-βCD (50 mM) in water and in different acids.

The results show a synergistic effect between acids and CDs. The solubility in presence of both acids (e.g. lactic acid) and, for example, HP-βCD (9626 μg/ml) is greater than that expected by adding the effects of CD and acid separately (76 and 2420 μg/ml). Contrary to the previous observation, the differences observed in the presence of CDs between the different acids (50 mM) can be explained by differences in the pH value of the solution: the pH values of the different solutions are 3.7, 3.6, 3.8 and 3.0, respectively, with citric, lactic, gluconic and phosphoric acids. The lower pH value of the phosphoric solution explains its better solubilizing effect. In this case, the pH is the most important factor.

At the same concentration, SBE$_7$-βCD allows to increase the solubility of miconazole at a higher level than HP-βCD.

^{1}H NMR spectroscopy was used to confirm the inclusion of miconazole in the CD cavity in an acidic medium. All measurement were made with βCD.

The ^{1}H NMR spectra between 3.0 and 5.5 ppm, reporting the CH protons of βCD in a pD3 phosphate buffer solution alone (A) or associated in the complex with miconazole (B) are shown in Fig. 6. Table 2 reports the chemical shift values of βCD protons in the free and complex state, as well as the differences between the signals of the free and included molecules.

The inclusion of a guest molecule into the βCD cavity clearly induces some changes in the ^{1}H NMR chemical shift values. Among the different detected signals, those corresponding to H3 and H5 protons are the most affected (H3 and H5 protons are located inside the cavity). This behavior is typical of the inclusion of aromatic molecules in the hydrophobic cavity of βCD (Djedaïni and Perly, 1991).

Significant changes in the chemical shift values were also observed in the miconazole spectrum for the complex state versus the free state. Table 3 reports the chemical shift values of miconazole. Because of the asymmetric carbon, miconazole has two enantiomers that can be differentiated in presence of CD. This differentiation is character-

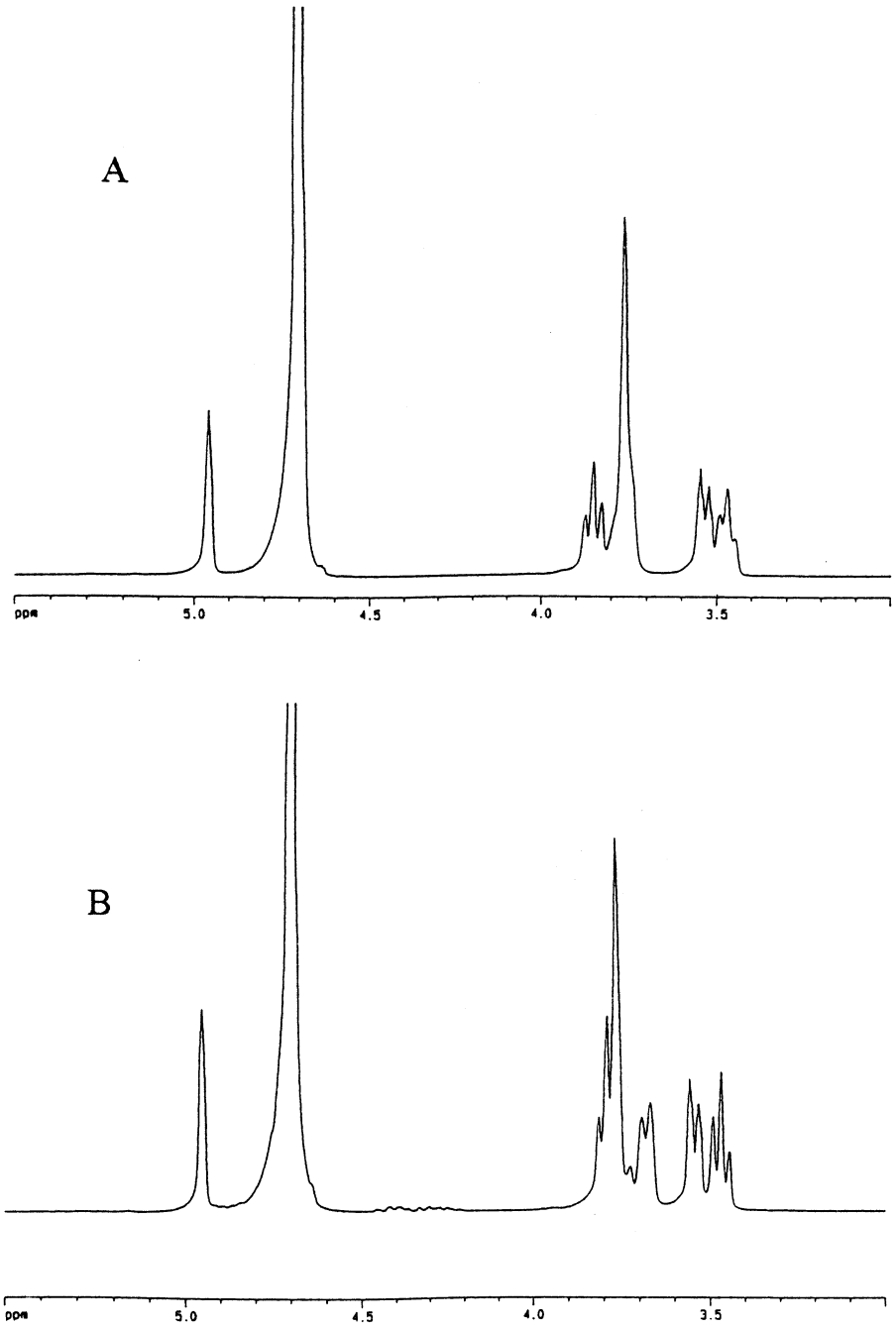

Fig. 6. ^{1}H NMR spectra (400.13 MHz) of (A) the βCD and (B) the miconazole–βCD complex in a pD3 phosphate buffer solution, between 3 and 5.5 ppm.

ized by a splitting of the chemical shift signal. From the obtained results, it can be seen that cycle II (Fig. 1) and the imidazole cycle are the most affected by the presence of β-CD. Cycle I only shows small chemical shift values differences. In the present state of our research, we think that

Table 2
Chemical shifts values (ppm) for the protons of βCD in the free and in the complex state

Proton	δ	δ_c	$\Delta\delta_c$
H1	4.9560	4.9516	0.0044
H2	3.5348	3.5457	−0.0109
H3	3.8501	3.7927	0.0574
H4	3.4694	3.4692	0.0002
H5	3.7628	3.6822	0.0806
H6	3.7628	3.7685	−0.0057

δ, chemical shift in ppm of the corresponding H of the cyclodextrin without miconazole; δ_c, chemical shift in ppm of the corresponding H of the cyclodextrin with miconazole; $\Delta\delta_c$, difference between δ and δ_c.

the imidazole cycle and cycle II are included in the CD cavity.

The stoichiometry of the complex was determined by the continuous variation method. Fig. 7 shows the continuous variation plots obtained for the most markedly affected proton of miconazole (H2). It shows a maximum at $r = 0.5$ and highly symmetrical shapes, indicating that a 1:1 complex is formed.

Table 3
Chemical shifts values (ppm) for the protons of miconazole in the free and in the complex state

Proton	δ	δ_c		$\Delta\delta_c$	
Imidazole					
H2	8.540	8.620	8.670	−0.080	−0.130
H4	7.255	7.338	7.352	−0.083	−0.097
H5	7.265	7.287	7.293	−0.022	−0.028
2.4-Dichlorophenyl cycles					
Cycle I					
H3	7.370	7.373	7.388	−0.003	−0.018
H4	7.200	7.210	7.210	−0.010	−0.010
H5	7.160	7.050	7.090	0.110	0.070
Cycle II					
H3′	7.490	7.537	7.550	−0.047	−0.060
H4′	7.325	7.426	7.469	−0.101	−0.144
H5′	7.320	7.468	7.515	−0.148	−0.195

δ, chemical shift in ppm of the corresponding H of the miconazole without cyclodextrin; δ_c, chemical shift in ppm of the corresponding H of the miconazole with cyclodextrin; $\Delta\delta_c$, difference between δ and δ_c.

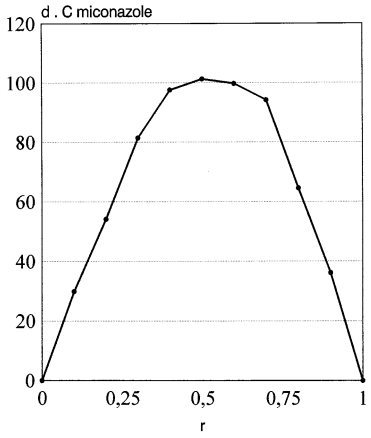

Fig. 7. Continuous variation plots obtained for the selected proton of miconazole (H2).

3.4. Development of the non-surfactant miconazole intravenous solution

A 100 mM HP-βCD–50 mM lactic acid (Table 4) or 50 mM SBE_7-βCD–50 mM lactic acid combination was chosen to formulate the parenteral solution, firstly because lactic acid is currently used in intravenous solutions and is already present in a marketed[1] solution and, secondly, because this solution has an acceptable pH value (around 3.8). This combination allows to solubilize more than 10 mg of miconazole per ml which is the concentration of the marketed solution. A solution of miconazole at a concentration of 10 mg/ml was prepared in purified water containing HP-βCD 100 mM and lactic acid 50 mM.

NaCl was added (4.5 mg/ml) to obtain an isoosmotic solution. This solution did not precipitate when diluted 5 or 10 times in NaCl 0.9% solution. The solution was stable to sterilization

Table 4
Composition of the miconazole parenteral solution

Miconazole	10.00 mg
HP-βCD	139.44 mg
Lactic acid	5.30 mg
NaCl	4.47 mg
Water for injection	ad 1 ml

[1] DAKTARIN® (Janssen-Cilag)

Table 5
Stability study of the parenteral solution of miconazole

Temperature (°C)	Miconazole content (mg/ml)						
	T_0	$T_{1\ week}$	$T_{2\ weeks}$	$T_{1\ month}$	$T_{2\ months}$	$T_{3\ months}$	$T_{6\ months}$
4	9.72	9.93	9.86	10.03	9.59	9.95	9.84
25	9.72	9.93	9.70	9.93	9.84	9.92	10.00
45	9.72	9.83	9.83	10.04	9.76	10.07	10.18

by saturated steam (121°C, 15 min) and did not contain endotoxins.

Stability studies at 4, 25 and 45°C showed that the solution with HP-βCD was stable for at least 6 months at these temperatures. As shown in Table 5, the miconazole content is between the limits 9.5–10.5 mg/ml. The pH value was also stable (3.8) and no precipitation occurred.

In conclusion, these results show that miconazole can be conveniently prepared in aqueous solution of HP-βCD (100 mM) or SBE_7-βCD (50 mM) and lactic acid (50 mM). This combination eliminates the need for including polyoxyl 35 castor oil in the parenteral dosage formulation with the potential advantage of fewer toxic reactions.

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