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International Journal of Pharmaceutics 264 (2003) 25–34
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Solubilization of Fluasterone in cosolvent/cyclodextrin combinations

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Received 14 January 2003; received in revised form 11 April 2003; accepted 7 July 2003

Abstract

The combined effect of cosolvent (methanol (MeOH), ethanol (EtOH), or *n*-propanol (*n*-PrOH)) and complexant hydroxypropyl-β-cyclodextrin (HPβCD) on the solubility of Fluasterone is evaluated and explained with a simple equation. The calculated constants in the equation not only quantitatively describe the dependence of drug solubility on cosolvent and ligand concentrations, but also explain the minima that are observed in the Fluasterone solubility versus cosolvent concentration curves at fixed HPβCD concentrations.

Keywords: Solubilization; Cosolvent; Cyclodextrin; Complexation; Mixed solvent; Fluasterone

1. Introduction

The solubility of a drug in aqueous media determines many aspects of its efficacy for delivery and absorption. Solubilization can be achieved by: pH control, cosolvency, complexation, micellization, or a combination effect of any of the above. Applying multiple techniques can be advantageous for drugs that cannot be optimally solubilized by a single technique. It also enables the use of a smaller amount of any single excipient. The combined use of cosolvency and complexation is a particularly interesting case.

The effect of cosolvents and complexants when used individually are well understood. Cosolvents work by reducing the hydrogen bond density of water and consequently its ability to "squeeze out" nonpolar solutes from aqueous systems. In other words, they increase the solubility of nonpolar drugs by reducing the polarity of the aqueous mixture. Inclusion ligands, such as the cyclodextrins, increase the drug solubility by reversibly incorporating the nonpolar portion of the drug into their nonpolar cavities.

Some researchers have reported that cosolvents decrease drug solubility in the complex (Loftsson et al., 1993; Ono et al., 2001), while others reported an increase (Zung, 1991; Savolainen et al., 1998; Loftsson and Petersen, 1998; Loftsson et al., 2001; Faucci and Mura, 2001). According to Connors (1997), at least five different explanatory hypotheses have been proposed for the effect of the solvent in drug-complexant systems. Some of these hypotheses attribute this effect to changes of the hydrophobic driving force for formation of the drug complex. Some attribute it to changes in the solvophobic characteristics on the medium. And still others proposed a decrease in the stoichiometric equilibrium with the addition of organic cosolvent.

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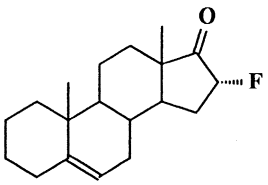
doi:10.1016/S0378-5173(03)00389-2

Li et al. (1999) reported that the solubility of Fluasterone, a new chemoprevention agent and a type 2 diabetes drug, is first decreased and then increased as ethanol is added to a solution containing hydroxypropyl-β-cyclodextrin. This study is aimed at quantitatively characterizing the effect of cosolvent size and polarity on the solubility of Fluasterone in the presence of complexation ligand. Methanol (MeOH), ethanol (EtOH), and *n*-propanol (*n*-PrOH) were chosen as the cosolvents because they are a series with increasing molecular size and reducing polarity.



Fluasterone

2. Materials and methods

2.1. Materials

Fluasterone (micronized) was provided by Aeson Therapeutics Inc. (Tucson, AZ). Hydroxypropyl-β-cyclodextrin (HPβCD, Trappsol®) with an average molecular weight of 1390 and an average degree of hydroxypropyl substitution at 4.4 was obtained from Cyclodextrin Technologies Development Inc. (Gainesville, FL). ACN (acetonitrile), MeOH, EtOH, EtOAc (ethyl acetate), and *n*-PrOH were of reagent or HPLC grade and purchased from Aldrich (Milwaukee, WI). All chemicals were used as received without further purification and the water was double-deionized.

2.2. Solubility determination

The solubility of Fluasterone in water was measured via the phase-solubility method followed by extraction. Sample bottles containing excess Fluasterone in 100 ml water were rotated for 6 days at room temperature on an end-over-end mechanical rotator (Glas-Col Laboratory Rotator, Terre Haute, IN). The saturated Fluasterone–water solutions were filtered with a 0.45 μm membrane, and then the filtrates were extracted three times with 10 ml of EtOAc. The organic phases were then combined and dried under nitrogen. The residue was redissolved into 2 ml of 75% ACN in water for HPLC analysis.

Similarly, the solubilities of Fluasterone in aqueous systems containing cosolvent, HPβCD, or combined cosolvent-HPβCD were measured by the phase-solubility method. Solvent systems of 0–88% (v/v) cosolvent and/or 0–20% (w/v) HPβCD in water were prepared by adding an exact amount of HPβCD into the water or cosolvent/water mixture. Saturated Fluasterone solutions were obtained by filtering excess Fluasterone from 2 ml of above solvent systems after the sample vials were rotated for 6 days. The diluted filtrates were analyzed by HPLC. All the samples were prepared and analyzed in duplicate.

2.3. HPLC analysis of Fluasterone

The HPLC assay method reported by Li et al. (1999) with a A Beckman Gold System (Fullerton, CA) was used. A Pinnacle 5 μm C8 amine column (150 cm × 4.6 mm, Restek, Bellefonte, PA) was used as the stationary phase with 75% ACN and 25% water as the mobile phase. The flow rate was controlled at 1.1 ml/min (125 Solvent Module) for 9 min. The sample injection volume was 100 μl (507 Autosampler) and the analytes were detected at 220 nm (168 Detector). The retention time of Fluasterone was 6.3 min. None of the solubilizing agents interfered with the assay.

3. Background

3.1. Fluasterone in water or cosolvent solutions

The solubility of Fluasterone in water, i.e. the intrinsic solubility, was determined by HPLC to be 0.000155 mM (0.045 μg/ml).

The exponential dependence of the solubility of nonpolar solutes on cosolvent concentration in a semi-aqueous solution is described in Eq. (1), which can be written in log-linear form as Eq. (2) (Yalkowsky et al., 1972; Yalkowsky and Rubino, 1985):

$$[D_{tot}] = [D_u]10^{\sigma[C]}, \quad (1)$$

$$\log[D_{tot}] = \log[D_u] + \sigma[C], \quad (2)$$

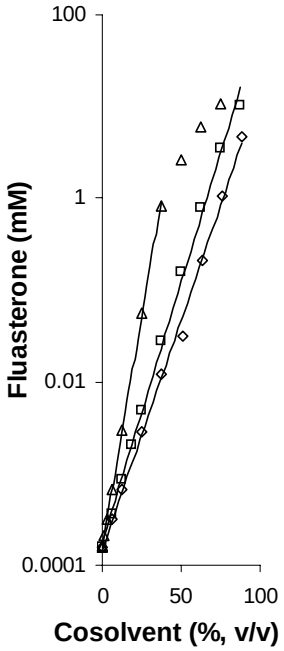


Fig. 1. Solubility of Fluasterone in cosolvent solutions. ◇, MeOH; □, EtOH; and △, *n*-PrOH.

where $[D_{tot}]$ is the total apparent drug solubility, $[D_u]$ is the intrinsic drug solubility, [C] is the cosolvent concentration, σ is the cosolvent solubilizing power for the solute.

Fig. 1 shows the solubility of Fluasterone in the cosolvent solutions. The solubility data in all tested concentrations of MeOH, EtOH, and up to 40% *n*-PrOH conform to the predictions of Eqs. (1) and (2). As expected, the solubility of Fluasterone increases least in MeOH (the most polar solvent) solution and most in *n*-PrOH (the least polar solvent) solution. The σ values are 0.19, 0.34, and 0.78 M^{-1} for MeOH, EtOH, and *n*-PrOH, respectively.

3.2. Fluasterone in complexant solution

The linear dependence of the solubility of nonpolar drug on HPβCD is described in Eq. (3):

$$[D_{tot}] = [D_u] + K_b[D_u][L], \quad (3)$$

where K_b is the equilibrium constant for the binary complex [DL] and [L] is the ligand concentration.

Fig. 2 shows the measured Fluasterone solubility in solutions of 0–20% HPβCD. The Fluasterone–HPβCD

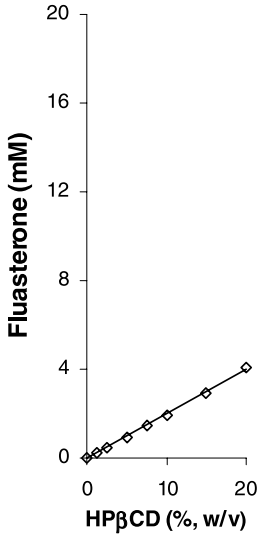


Fig. 2. Solubility of Fluasterone in HPβCD solution.

binary complex equilibrium constant calculated from the slope is $1.8 \times 10^5\ M^{-1}$. Since the concentration of binary drug ligand complex is given by the last term in Eq. (3) ($K_b[D_u][L]$), there is a linear increase in total drug solubility with increasing of ligand concentration.

3.3. Fluasterone in solutions of cosolvent and HPβCD

The presence of ternary complex of nonpolar drug with cosolvent in the β-cyclodextrin cavity has been reported by many researchers (Schuette et al., 1993; Milewski et al., 1998; Evans and Partyka, 2000). On the other hand, some researchers (Ono et al., 2001; Zhao et al., 2002; Yang et al., 2003) have reported competition between guest drug molecule and competitor to complex with the cyclodextrin when the drug and the competitor have similar polarity and molecular size. When Fluasterone dissolves in a solvent system of either MeOH, EtOH, or *n*-PrOH with HPβCD, it is likely to form a ternary complex because each cosolvent is much smaller and are much more polar than Fluasterone. Some of the Fluasterone will dissolve in the medium as free drug, [D], some will dissolve by inclusion in the ligand cavity as binary complex, [DL], and some will dissolve by inclusion with the cosolvent in the ligand cavity as a ternary complex, [DLC].

The total solubility of Fluasterone is the sum of the concentrations of all three of these drug species, i.e.

$$[D_{tot}] = [D] + [DL] + [DLC]. \quad (4)$$

The concentrations of these species are described by Eqs. (5)–(7)

$$[D] = [D_u]10^{\sigma[C]}, \quad (5)$$

$$[DL] = (K_b 10^{-\rho_b[C]})([D_u]10^{\sigma[C]})[L] = K_b[D_u][L]10^{(\sigma-\rho_b)[C]}, \quad (6)$$

$$[DLC] = (K_t 10^{-\rho_t[C]})([D_u]10^{\sigma[C]})[L][C] = K_t[D_u][L][C]10^{(\sigma-\rho_t)[C]}, \quad (7)$$

where K_t is the equilibrium constant for the ternary complex [DLC], ρ_b is the destabilizing power of the cosolvent for the binary complex [DL], i.e. the effect of the cosolvent on K_b as described by Li et al. (1999). ρ_t is the destabilizing power of the cosolvent for the ternary complex [DLC], i.e. the effect of the cosolvent on K_t as described by Li et al. (1999).

Note that the free drug concentration in Eq. (5) is equal to the total drug concentration in Eq. (1), when only cosolvent is added into the system. Eqs. (6) and (7) account for two effects of the cosolvent on complex formation. First, cosolvent addition favors complexation because it provides more solute in the solution by $10^{\sigma[C]}$. Second, cosolvent addition disfavors complexation because it reduces the driving force for solute incorporation into the CD cavity by $10^{-\rho_b[C]}$ or $10^{-\rho_t[C]}$. Note that ρ_t can be either positive or negative depending upon whether the cosolvent destabilizes or stabilizes the ternary complex.

In order to obtain the total drug concentration, Eqs. (5)–(7) are inserted into Eq. (4) to give Eq. (8):

$$[D_{tot}] = [D_u]10^{\sigma[C]} + K_b[D_u][L]10^{(\sigma-\rho_b)[C]} + K_t[D_u][L][C]10^{(\sigma-\rho_t)[C]}. \quad (8)$$

Eq. (8) expresses the total drug solubility as a function of both the ligand and cosolvent concentrations with five constants for each system: σ is the cosolvent solubilization power; K_b and K_t are the formation constants for the binary and ternary complexes, respectively; ρ_b and ρ_t are the cosolvent destabilizing powers for the binary complex and ternary complexes, respectively.

Eq. (8) can be rearranged to give Eq. (9), which expresses the total solubility as a linear function of ligand concentration:

$$[D_{tot}] = [D_u]10^{\sigma[C]} + \{K_b[D_u]10^{(\sigma-\rho_b)[C]} + K_t[D_u][C]10^{(\sigma-\rho_t)[C]}\}[L]. \quad (9)$$

4. Results and discussion

The measured solubilities of Fluasterone in aqueous systems of MeOH and HPβCD, EtOH and HPβCD, and *n*-PrOH and HPβCD are plotted as symbols in Fig. 3 as a function of cosolvent concentration at several fixed HPβCD concentration (from 20 to 0%, w/v). The data strings are organized in order of reducing HPβCD concentration from the top to the bottom in each system. The symbols for the various HPβCD concentrations are listed in Table 1.

The experimental data in Fig. 3 were used to obtain the constants in Eq. (8) via nonlinear multiple regression. The values of the constants for each water-cosolvent-HPβCD-Fluasterone system are listed into Table 2.

The solubility of Fluasterone can be calculated by Eq. (8) under any chosen cosolvent-ligand concentrations, once the constants are generated as in Table 2. The calculated solubilities are indicated as the solid lines in Fig. 3. Clearly, they are in good agreement with the experimental data. The average absolute error (AAE) for the logarithms of the measured and calculated data are 0.05, 0.05, and 0.17 for the MeOH, EtOH, and *n*-PrOH systems, respectively.

Table 1
The symbols and HPβCD concentrations of the data strings in Fig. 3

Symbol	Concentration of HPβCD (%, w/v)		
	A	B	C
△	20	20	20
□	15	15	
*	10	10	10
○		7.5	
×	5	5	5
+	2.5	2.5	2.5
−	1.25	1.25	1.25
◇	0	0	0

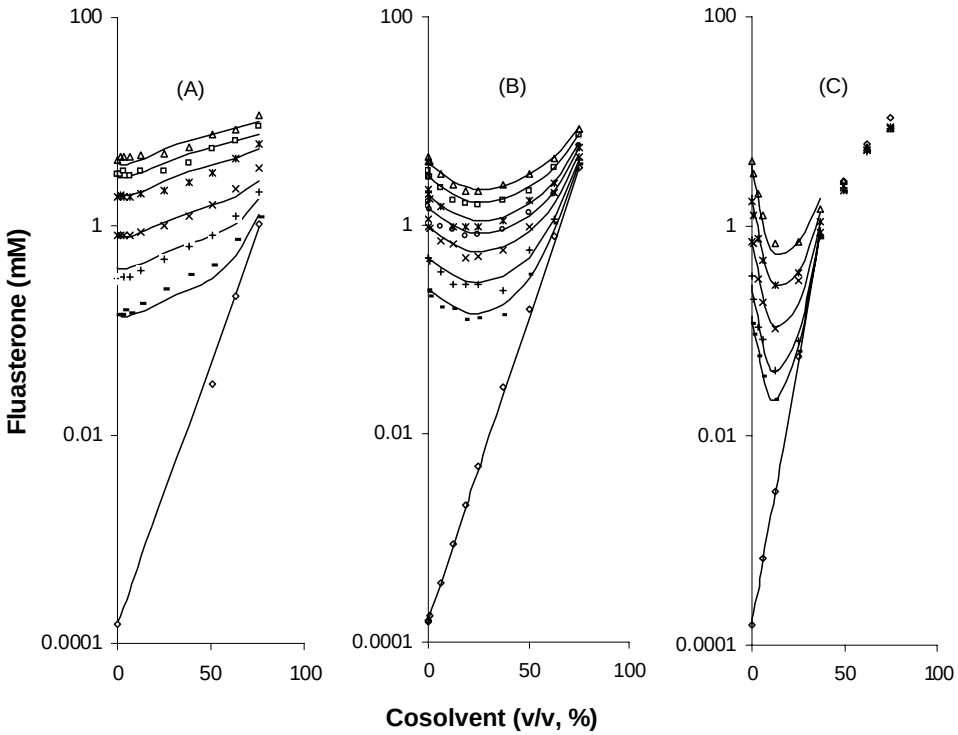


Fig. 3. Dependence of Fluasterone solubility on cosolvent in cosolvent-HPβCD systems. (A) MeOH and HPβCD; (B) EtOH and HPβCD; (C) *n*-PrOH and HPβCD.

Interestingly, the solubility of Fluasterone decreases and then increases as the cosolvent concentration increases. This effect is quantitatively explained in Eq. (8) by the changes in the concentration of each drug component. As cosolvent composition increases, the free drug, [D], exponentially increases as indicated in Eq. (5). However, the changes of the other two drug components could be in either direction. As indicated in Eq. (6), the binary drug–ligand complex, [DL], can exponentially increase, decrease, or it can remain steady with increasing cosolvent composition, depending on whether the term $(\sigma - \rho_b)$ is greater than, less than, or equal to zero. Similarly, the changes of the ternary drug–ligand–cosolvent complex, [DLC], is described by Eq. (7). This change can also go either direction with increasing cosolvent composition, depending on whether the term $(\sigma - \rho_t)$ is positive, negative, or equal to zero. The summation of the three drug components determines the shape of the total drug solubility curve. This model explains the observed reduced and then increased solubility of Fluasterone in the three tested systems. It also explains why some researchers observed a decrease in total drug solubility with increasing cosolvent composition, while others observed an increase.

Table 2
Parameters of the solvent systems

Parameter	MeOH	EtOH	*n*-PrOH
σ (M^{-1})	0.19	0.34	0.78
ρ_b (M^{-1})	0.30	0.52	1.58
ρ_t (M^{-1})	0.21	0.34	0.79
K_b (M^{-1})	1.8E+05	1.8E+05	1.8E+05
K_t (M^{-2})	4.3E+04	1.4E+04	9.1E+03

Comparing the three graphs in Fig. 3, the most polar and smallest size cosolvent, MeOH, gives the least reduction in total drug solubility. The calculated parameter (K_t) in Table 2 indicates that MeOH has the greatest ability to form a drug–ligand–cosolvent complex. This could be attributed to its greater ability to fit into the spaces within the HPβCD cavities that are not

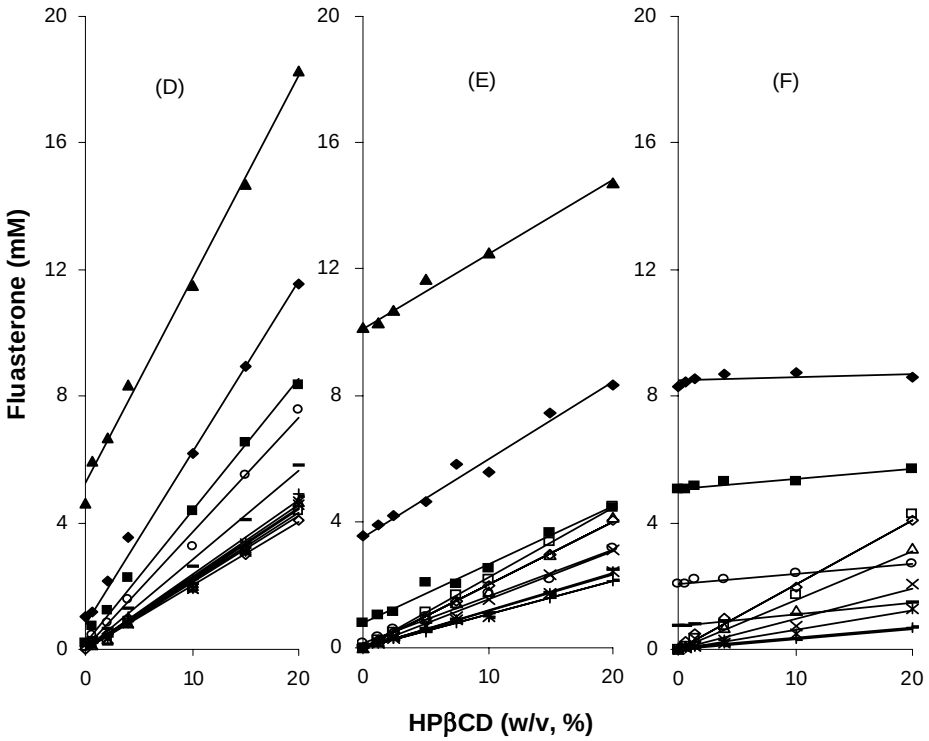


Fig. 4. Dependence of Fluasterone solubility on complexation ligand in cosolvent-HPβCD systems. (D) MeOH and HPβCD; (E) EtOH and HPβCD; (F) *n*-PrOH and HPβCD.

occupied by drug. The increase of the ternary complex overcomes the loss of the binary complex to give an increase in total drug solubility.

The least polar and largest cosolvent, *n*-PrOH, produces the most pronounced reduction in solubility because it reduces the binary drug–ligand complex the most. The calculated parameter (K_t) in Table 2 indicates that *n*-PrOH has the least ability to form a ternary complex. The main contribution of the increased drug solubility is the free drug as a result of the high cosolvent solubilization power (σ) of *n*-PrOH.

The solubilities of Fluasterone are shown as a function of HPβCD concentration in Fig. 4, which contains the rearranged data of Fig. 3. Each data string represents a fixed cosolvent concentration and is organized in each graph in order of reducing cosolvent concentration (from 88 to 0%, v/v) from the top to the bottom. The cosolvent concentrations and symbols are listed in Table 3. The linear dependence of Fluasterone solubility on HPβCD in each cosolvent system is in good agreement with Eq. (9). Note, that if the data sets in graphs D, E, and F are vertically connected at 20% HPβCD, they correspond to the data strings on the top of graphs A, B, and C, respectively. The vertical data sets from right to left in Fig. 4 correspond to horizontal

Table 3
The symbols and cosolvent concentration of the data strings in Fig. 4

Symbol	Concentration of cosolvent (%, v/v)		
	D (MeOH)	E (EtOH)	F (*n*-PrOH)
▲	88.6	87.7	
◆	75.9	75.2	74.6
■	63.2	62.3	62.2
○	50.6	50.1	49.8
−	37.9	37.6	37.3
+	25.3	25.1	24.9
–	12.7	18.8	12.4
*	6.3	12.5	6.2
×	3.2	6.3	3.1
△	1.3	1.0	1.2
□	0.3	0.2	0.3
◇	0.0	0.0	0.0

data strings from top to bottom in Fig. 3. The measured solubility data are provided in the appendix.

5. Conclusions

Fluasterone solubility in combined cosolvent and complexant solutions is the summation of the free drug in the semi-aqueous media, the drug–ligand binary complex, and the drug–ligand–cosolvent ternary complex. The presence of cosolvent reduces the overall polarity of the aqueous media while it has a tendency to be included in a ternary complex based on its small molecular size. The simple Eq. (8) can explain a decrease, increase, or decrease followed by increase in drug solubility with the addition of a cosolvent to a HPβCD and drug solution. It accounts for the effect of cosolvent size and polarity on the solubilization of the drug, the destabilization of drug–ligand complex and the formation of a ternary drug–ligand–cosolvent complex.

Appendix A

Solvent system*	Fluasterone solubility (mM)
Water	1.55E−04
1.25% HPβCD	0.23
2.5% HPβCD	0.49
5% HPβCD	0.96
7.5% HPβCD	1.49
10% HPβCD	1.96
15% HPβCD	2.93
20% HPβCD	4.04
6.3% MeOH	3.20E−04
12.7% MeOH	6.61E−04
25.3% MeOH	2.81E−03
37.9% MeOH	1.19E−02
50.6% MeOH	3.09E−02
63.2% MeOH	0.21
75.9% MeOH	1.04
88.6% MeOH	4.60
6.3% EtOH	3.67E−04
12.5% EtOH	8.72E−04
18.8% EtOH	2.07E−03
25.1% EtOH	4.91E−03
37.6% EtOH	2.77E−02
50.1% EtOH	0.16
62.7% EtOH	0.80
75.2% EtOH	3.54
87.7% EtOH	10.15
0.3% *n*-PrOH	1.64E−04
1.2% *n*-PrOH	2.08E−04
3.1% *n*-PrOH	3.24E−04
6.2% *n*-PrOH	6.72E−04
12.4% *n*-PrOH	2.91E−03
24.9% *n*-PrOH	5.72E−02
37.3% *n*-PrOH	0.83
49.8% *n*-PrOH	2.66
62.2% *n*-PrOH	6.09
74.6% *n*-PrOH	10.83
0.3% MeOH & 1.25% HPβCD	0.16
1.3% MeOH & 1.25% HPβCD	0.14
3.2% MeOH & 1.25% HPβCD	0.14
6.3% MeOH & 1.25% HPβCD	0.16
12.7% MeOH & 1.25% HPβCD	0.15
25.3% MeOH & 1.25% HPβCD	0.18
37.9% MeOH & 1.25% HPβCD	0.25
50.6% MeOH & 1.25% HPβCD	0.34
63.2% MeOH & 1.25% HPβCD	0.42
75.9% MeOH & 1.25% HPβCD	0.74
88.6% MeOH & 1.25% HPβCD	1.19
0.3% MeOH & 2.5% HPβCD	0.44
1.3% MeOH & 2.5% HPβCD	0.32
3.2% MeOH & 2.5% HPβCD	0.32
6.3% MeOH & 2.5% HPβCD	0.33
12.7% MeOH & 2.5% HPβCD	0.32
25.3% MeOH & 2.5% HPβCD	0.38
37.9% MeOH & 2.5% HPβCD	0.48
50.6% MeOH & 2.5% HPβCD	0.64
63.2% MeOH & 2.5% HPβCD	0.83
75.9% MeOH & 2.5% HPβCD	1.25
88.6% MeOH & 2.5% HPβCD	2.16
0.3% MeOH & 5% HPβCD	0.96
1.3% MeOH & 5% HPβCD	0.82
3.2% MeOH & 5% HPβCD	0.82
6.3% MeOH & 5% HPβCD	0.83
12.7% MeOH & 5% HPβCD	0.82
25.3% MeOH & 5% HPβCD	0.89
37.9% MeOH & 5% HPβCD	1.03

Appendix A (*Continued*)

Appendix A (*Continued*)

Solvent system*	Fluasterone solubility (mM)
50.6% MeOH & 5% HPβCD	1.26
63.2% MeOH & 5% HPβCD	1.59
75.9% MeOH & 5% HPβCD	2.24
88.6% MeOH & 5% HPβCD	3.56
0.3% MeOH & 10% HPβCD	1.96
1.3% MeOH & 10% HPβCD	1.91
3.2% MeOH & 10% HPβCD	1.99
6.3% MeOH & 10% HPβCD	1.92
12.7% MeOH & 10% HPβCD	1.92
25.3% MeOH & 10% HPβCD	2.06
37.9% MeOH & 10% HPβCD	2.20
50.6% MeOH & 10% HPβCD	2.62
63.2% MeOH & 10% HPβCD	3.23
75.9% MeOH & 10% HPβCD	4.40
88.6% MeOH & 10% HPβCD	6.21
0.3% MeOH & 15% HPβCD	3.05
1.3% MeOH & 15% HPβCD	3.10
3.2% MeOH & 15% HPβCD	3.19
6.3% MeOH & 15% HPβCD	3.32
12.7% MeOH & 15% HPβCD	3.07
25.3% MeOH & 15% HPβCD	3.32
37.9% MeOH & 15% HPβCD	3.40
50.6% MeOH & 15% HPβCD	4.08
63.2% MeOH & 15% HPβCD	5.48
75.9% MeOH & 15% HPβCD	6.52
88.6% MeOH & 15% HPβCD	8.92
0.3% MeOH & 20% HPβCD	4.04
1.3% MeOH & 20% HPβCD	4.36
3.2% MeOH & 20% HPβCD	4.65
6.3% MeOH & 20% HPβCD	4.69
12.7% MeOH & 20% HPβCD	4.58
25.3% MeOH & 20% HPβCD	4.82
37.9% MeOH & 20% HPβCD	4.92
50.6% MeOH & 20% HPβCD	5.82
63.2% MeOH & 20% HPβCD	7.54
75.9% MeOH & 20% HPβCD	8.36
88.6% MeOH & 20% HPβCD	11.53
0.2% EtOH & 1.25% HPβCD	0.23
1.0% EtOH & 1.25% HPβCD	0.21
6.3% EtOH & 1.25% HPβCD	0.16
12.5% EtOH & 1.25% HPβCD	0.15
18.8% EtOH & 1.25% HPβCD	0.12
25.1% EtOH & 1.25% HPβCD	0.13
37.6% EtOH & 1.25% HPβCD	0.14

Appendix A (*Continued*)

Solvent system*	Fluasterone solubility (mM)
50.1% EtOH & 1.25% HPβCD	0.34
62.7% EtOH & 1.25% HPβCD	1.04
75.2% EtOH & 1.25% HPβCD	3.87
87.7% EtOH & 1.25% HPβCD	10.29
0.2% EtOH & 2.5% HPβCD	0.48
1.0% EtOH & 2.5% HPβCD	0.46
6.3% EtOH & 2.5%HPβCD	0.36
12.5% EtOH & 2.5% HPβCD	0.27
18.8% EtOH & 2.5%HPβCD	0.27
25.1% EtOH & 2.5% HPβCD	0.27
37.6% EtOH & 2.5%HPβCD	0.24
50.1% EtOH & 2.5%HPβCD	0.57
62.7% EtOH & 2.5%HPβCD	1.14
75.2% EtOH & 2.5%HPβCD	4.19
87.7% EtOH & 2.5%HPβCD	10.69
0.2% EtOH & 5% HPβCD	1.15
1.0% EtOH & 5% HPβCD	0.92
6.3% EtOH & 5%HPβCD	0.71
12.5% EtOH & 5% HPβCD	0.67
18.8% EtOH & 5%HPβCD	0.49
25.1% EtOH & 5% HPβCD	0.51
37.6% EtOH & 5%HPβCD	0.57
50.1% EtOH & 5%HPβCD	0.98
62.7% EtOH & 5%HPβCD	2.08
75.2% EtOH & 5%HPβCD	4.61
87.7% EtOH & 5%HPβCD	11.67
0.2% EtOH & 7.5% HPβCD	1.67
1.0% EtOH & 7.5% HPβCD	1.42
6.3% EtOH & 7.5%HPβCD	0.98
12.5% EtOH & 7.5% HPβCD	0.91
18.8% EtOH & 7.5%HPβCD	0.79
25.1% EtOH & 7.5% HPβCD	0.80
37.6% EtOH & 7.5%HPβCD	0.89
50.1% EtOH & 7.5%HPβCD	1.31
62.7% EtOH & 7.5%HPβCD	2.01
75.2% EtOH & 7.5%HPβCD	5.79
0.2% EtOH & 10% HPβCD	2.18
1.0% EtOH & 10% HPβCD	1.78
6.3% EtOH & 10%HPβCD	1.53
12.5% EtOH & 10% HPβCD	0.98
18.8% EtOH & 10%HPβCD	0.96
25.1% EtOH & 10% HPβCD	0.98
37.6% EtOH & 10%HPβCD	1.09
50.1% EtOH & 10%HPβCD	1.74

Appendix A (*Continued*)

Solvent system*	Fluasterone solubility (mM)
62.7% EtOH & 10%HPβCD	2.50
75.2% EtOH & 10%HPβCD	5.54
87.7% EtOH & 10%HPβCD	12.51
0.2% EtOH & 15% HPβCD	3.33
1.0% EtOH & 15% HPβCD	2.95
6.3% EtOH & 15%HPβCD	2.31
12.5% EtOH & 15% HPβCD	1.72
18.8% EtOH & 15%HPβCD	1.62
25.1% EtOH & 15% HPβCD	1.58
37.6% EtOH & 15%HPβCD	1.71
50.1% EtOH & 15%HPβCD	2.16
62.7% EtOH & 15%HPβCD	3.62
75.2% EtOH & 15%HPβCD	7.45
0.2% EtOH & 20% HPβCD	4.46
1.0% EtOH & 20% HPβCD	4.16
6.3% EtOH & 20%HPβCD	3.10
12.5% EtOH & 20% HPβCD	2.40
18.8% EtOH & 20%HPβCD	2.13
25.1% EtOH & 20% HPβCD	2.13
37.6% EtOH & 20%HPβCD	2.45
50.1% EtOH & 20%HPβCD	3.15
62.7% EtOH & 20%HPβCD	4.45
75.2% EtOH & 20%HPβCD	8.33
87.7% EtOH & 20%HPβCD	14.71
0.3% *n*-PrOH & 1.25% HPβCD	0.12
1.2% *n*-PrOH & 1.25% HPβCD	9.21E−02
3.1% *n*-PrOH & 1.25% HPβCD	5.78E−02
6.2% *n*-PrOH & 1.25% HPβCD	3.62E−02
12.4% *n*-PrOH & 1.25% HPβCD	2.18E−02
24.9% *n*-PrOH & 1.25% HPβCD	6.22E−02
37.3% *n*-PrOH & 1.25% HPβCD	0.75
49.8% *n*-PrOH & 1.25% HPβCD	2.09
62.2% *n*-PrOH & 1.25% HPβCD	5.06
74.6% *n*-PrOH & 1.25% HPβCD	8.48
0.3% *n*-PrOH & 2.5% HPβCD	0.33
1.2% *n*-PrOH & 2.5% HPβCD	0.20
3.1% *n*-PrOH & 2.5% HPβCD	0.11
6.2% *n*-PrOH & 2.5% HPβCD	8.42E−02
12.4% *n*-PrOH & 2.5% HPβCD	4.20E−02
24.9% *n*-PrOH & 2.5% HPβCD	8.06E−02
37.3% *n*-PrOH & 2.5% HPβCD	0.80
49.8% *n*-PrOH & 2.5% HPβCD	2.20
62.2% *n*-PrOH & 2.5% HPβCD	5.17
74.6% *n*-PrOH & 2.5% HPβCD	8.55

Appendix A (*Continued*)

Solvent system*	Fluasterone solubility (mM)
0.3% *n*-PrOH & 5% HPβCD	0.72
1.2% *n*-PrOH & 5% HPβCD	0.68
3.1% *n*-PrOH & 5% HPβCD	0.32
6.2% *n*-PrOH & 5% HPβCD	0.19
12.4% *n*-PrOH & 5% HPβCD	0.11
24.9% *n*-PrOH & 5% HPβCD	0.30
37.3% *n*-PrOH & 5% HPβCD	0.86
49.8% *n*-PrOH & 5% HPβCD	2.23
62.2% *n*-PrOH & 5% HPβCD	5.32
74.6% *n*-PrOH & 5% HPβCD	8.70
0.3% *n*-PrOH & 10% HPβCD	1.72
1.2% *n*-PrOH & 10% HPβCD	1.25
3.1% *n*-PrOH & 10% HPβCD	0.76
6.2% *n*-PrOH & 10% HPβCD	0.47
12.4% *n*-PrOH & 10% HPβCD	0.28
24.9% *n*-PrOH & 10% HPβCD	0.36
37.3% *n*-PrOH & 10% HPβCD	1.09
49.8% *n*-PrOH & 10% HPβCD	2.40
62.2% *n*-PrOH & 10% HPβCD	5.31
74.6% *n*-PrOH & 10% HPβCD	8.75
0.3% *n*-PrOH & 20% HPβCD	4.26
1.2% *n*-PrOH & 20% HPβCD	3.20
3.1% *n*-PrOH & 20% HPβCD	2.05
6.2% *n*-PrOH & 20% HPβCD	1.28
12.4% *n*-PrOH & 20% HPβCD	0.69
24.9% *n*-PrOH & 20% HPβCD	0.70
37.3% *n*-PrOH & 20% HPβCD	1.47
49.8% *n*-PrOH & 20% HPβCD	2.69
62.2% *n*-PrOH & 20% HPβCD	5.72
74.6% *n*-PrOH & 20% HPβCD	8.61

* Cosolvent concentration in water is presented as volume to volume ratio (v/v, %), HPβCD concentration in water is presented as weight to volume ratio (w/v, %).

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