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Stability studies on steroidal drug/β-cyclodextrin kneaded systems

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Summary

6-Methylenandrosta-1,4-diene-3,17-dione (FCE24304), an aromatase inhibitor, is characterised not only by poor solubility in aqueous media and slow dissolution rate, but also by low chemical stability due to possessing conjugate double bonds. Several approaches have already been evaluated for improving both the biopharmaceutical properties and chemical stability of drugs in a particular way by the use of β-cyclodextrin. An accelerated short-term stability study on FCE24304/β-cyclodextrin kneaded systems, prepared using two different molar ratios, was carried out and the systems evaluated by HPLC, DSC and X-ray diffractometry techniques and correlated with dissolution rate behaviour. A tablet formulation with a 1:2 molar ratio of the FCE24304/β-cyclodextrin complex is now under development.

Introduction

One of the most interesting properties of cyclodextrins is their ability to improve the physico-chemical stability of a number of molecules in both solution and the solid state (Duchene et al., 1987), however, as reported in the literature, in the solid state, the association of drugs with β-cyclodextrins in some cases fails to improve their stability (Terada et al., 1983).

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In the search for an oral dosage form of 6-methylenandrosta-1,4-diene-3,17-dione (FCE 24304), a new synthetic antitumoral steroid in the class of aromatase inhibitors (Giudici et al., 1988), we have decided to develop a drug/β-cyclodextrin system in order to improve both its poor physico-pharmaceutical properties, e.g., low solubility in aqueous media and slow dissolution rate, and the chemical stability of the drug. As reported previously (Torricelli et al., 1990), we have evaluated various techniques of producing the complexes, such as cogrinding, kneading, coprecipitation and freeze-drying, and examined the feasibility of formulating a coground FCE24304/β-cyclodextrin system by performing an acceler-

ated short-term stability study (Torricelli et al., 1991). We have also carried out a short-term stability study under stress conditions on both 1 : 1 and 1 : 2 mol/mol drug/β-cyclodextrin kneaded systems. We have found that kneading is the easiest and more reproducible technique for obtaining the inclusion complex and, with the aim of developing a pharmaceutical dosage form of FCE24304 with β-cyclodextrin, we have monitored the chemical and physical stability of this more soluble, rapidly dissolving system. Recently, we have developed a tablet formulation which provides positive confirmation of the stability data.

Materials and Methods

Materials

Pure crystallised 6-methylenandrosta-1,4-diene-3,17-dione (FCE24304, Farmitalia Carlo Erba, Chemical R.&D., m.p. 195 °C) and β-cyclodextrin as provided by the supplier (Spad Roquette, batch 409886) were used.

Preparation of kneaded systems

1 : 1 mol/mol kneaded system : equimolar quantity of drug and β-cyclodextrin (1 : 4.29 w/w) were cosieved and tumble mixed. Inclusion was achieved by kneading the above-mentioned mixtures in a mortar with an adequate amount of water. The paste was worked for 30 min and then dried at 35 °C under vacuum to constant weight. The product was then sieved and tumble mixed again.

The 1 : 2 mol/mol kneaded product was obtained using the same technique as described above, but with different quantities of drug and β-cyclodextrin (1 : 8.58 w/w).

Storage conditions

Samples of the kneaded systems were stored at 35 and 55 °C sealed in vials (glass type I); under the other storage conditions (25 °C + 80% R.H.; 25 °C + 90% R.H.; 35 °C + 80% R.H.) the powder was spread on glass disks in order to expose a greater surface area to the moisturised atmosphere.

Chemical stability

The degradation products were detected by HPLC (SP8770 Knauer detector, 245 nm; Partisphere 5 μm; water/acetonitrile 60 : 40 v/v; 1 ml/min flow rate), and calculated by internal normalisation.

X-ray diffraction measurements

X-ray powder diffractograms were obtained using a Siemens D500TT diffractometer with CuKα as radiation source and the detector positron-sensitive (PSPC).

DSC measurements

Thermal analyses were carried out using a Mettler TA3000 system equipped with a DSC20 cell operating under nitrogen flow (50 ml/min). The heating rate was 10 °C/min.

Dissolution studies

Dissolution rate tests were carried out at 37 °C and 150 rpm in 900 ml phosphate buffer pH 7.4 according to USP XXII, no. 2, paddle method under sink conditions (maximum drug concentration 50 μg/ml). The drug concentration was detected by UV analysis (Philips PU8-7000) on filtered (Versapor 0.45 μm membranes) and diluted samples at 250 nm ($E^{1\%}_{1\,cm} = 496.5$).

Tablet formulation and preparation

Excipients used for tablets were microcrystalline cellulose (FMC), sodium starch glycolate (Mendell) and magnesium stearate (Farmitalia Carlo Erba).

The tablets were made up with FCE24304/β-cyclodextrin (1 : 2 mol/mol) kneaded system (193.7 mg, equivalent to 20 mg as drug substance), β-cyclodextrin (6.3 mg), sodium starch glycolate (17.7 mg), microcrystalline cellulose (10 mg) and magnesium stearate (2.3 mg). The correct amount of excipients, after sieving through a 45-mesh screen, was mixed with the kneaded system and the mixture was then directly compressed using a rotary tabletting machine equipped with 9 mm round concave punches. The tablets were packaged into amber glass bottles sealed with an aluminium-polyethylene snap-cap and stored for accelerated stability studies.

Results and Discussion

Thermal analyses of the FCE24304/β-cyclodextrin 1:2 mol/mol system demonstrate the complete disappearance of the melting event due to the drug: this indicates that an inclusion compound was formed when using such a technique. On the other hand, the DSC pattern of the 1:1 mol/mol system shows a thermal event at the melting temperature of the drug with a melting enthalpy corresponding to about 50% of that of the pure drug (Fig. 1). We can hypothesise that, since the complex between FCE24304 and β-cyclodextrin is in the ratio 1:2, as previously demonstrated (Torricelli et al., 1990), only half of the drug used is able to form an inclusion compound, while the other 50% remains as crystalline free drug in an intimate mixture with β-cyclodextrin. For the 1:1 mol/mol system, the thermal behaviour was unaffected by moist storage, but was modified by high temperatures (Figs 2 and 3): in fact, after 3 months at 55 °C, the melting event due to the drug disappears and a broader peak at about 180 °C becomes visible.

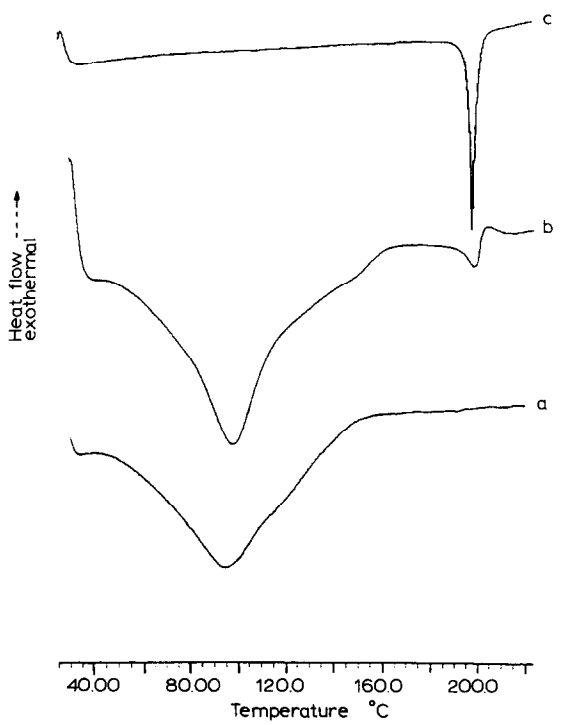

Fig. 1. DSC of (a) FCE24304/β-cyclodextrin 1:2 mol/mol kneaded system; (b) FCE24304/β-cyclodextrin 1:1 mol/mol kneaded system; (c) FCE24304 (parent drug).

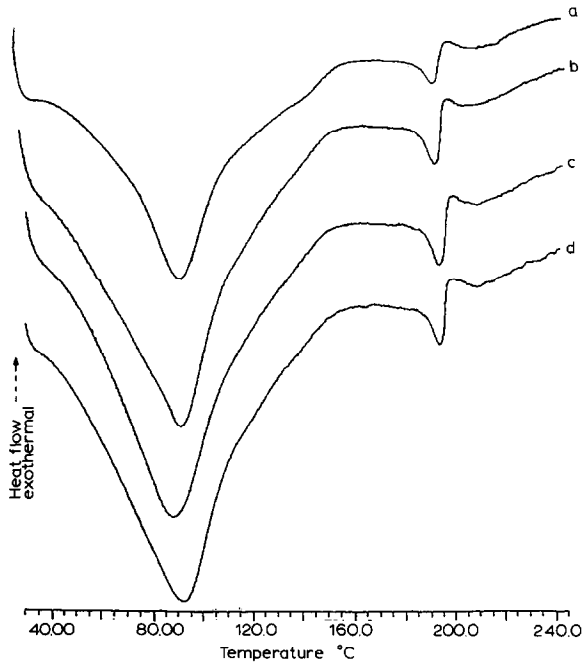

Fig. 2. DSC of FCE24304/β-cyclodextrin 1:1 mol/mol kneaded systems: (a) initial; (b) 3 months at 35 °C+80% R.H. storage; (c) 3 months at 25 °C+90% R.H. storage; (d) 3 months at 35 °C+80% R.H. storage.

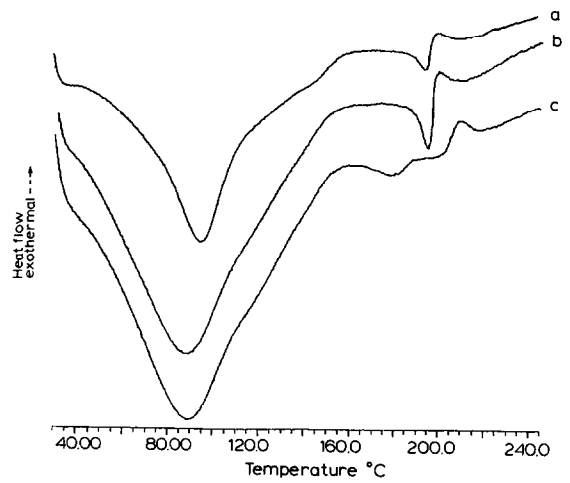

Fig. 3. DSC of FCE24304/β-cyclodextrin 1:1 mol/mol kneaded systems: (a) initial; (b) 3 months at 35 °C storage; (c) 3 months at 55 °C storage.

As shown in Table 1, the residual crystallinity of the drug itself remains unchanged, except for storage at 55 °C. On the other hand, we observed no visible change also at 55 °C for the 1:2 mol/mol system (Fig. 4 and Table 1).

Furthermore, DSC has been shown to be inappropriate as an analytical technique for evaluating the crystallinity pattern or recrystallization of complexes, X-ray diffractometry being more useful and predictive. In fact, DSC can only demonstrate whether any interactions occur between drug and β-cyclodextrin. It is of note that water vapour induces no detectable difference in FCE24304/β-cyclodextrin systems regarding both the thermal behaviour and chemical strength, although the water content of the two batches increases until the equilibrium content for cyclodextrin itself is attained.

The same differences were also observed (Table 2) on determination of the quantity of degradation products (percent of normalised area) after 1 and 3 months under various conditions of storage: only at 55 °C was there a significant increase in by-products in the 1:1 mol/mol system. These data are consistent with the hypothesis mentioned above: in the solid state the molecular ratio of the steroid/β-cyclodextrin complex is 1:2, therefore in the 1:1 system the rate of degradation vs the untreated drug is slow because only 50% of the drug is free and able to undergo degradation while the remaining 50% is protected in the β-cyclodextrin cavity. The results obtained by X-ray diffractographic investigation confirm these findings: storage at high temperature does not modify the diffractographic pattern of the 1:1 mol/mol system, while high moisture content does induce change (Fig. 5). The new pattern is comparable to that of the 1:2 mol/mol complex (Fig. 6). No significant changes were found with the 1:2 mol/mol system, except for a slight increase in the percentage of crystallinity.

TABLE 1

Degree of crystallinity as determined by DSC of samples stored under different conditions of temperature and humidity

Storage conditions	Degree of crystallinity (%)			
	Initial	15 days	1 month	3 months
1:1 mol/mol system				
–	49			
35 °C			45	45
55 °C			44	21 [a]
25 °C+80% R.H.		42	46	43
25 °C+90% R.H.		45	42	42
35 °C+80% R.H.		46	46	43
1:2 mol/mol system				
–	n.d.			
35 °C				n.d.
55 °C				n.d.
25 °C+80% R.H.		n.d.	n.d.	n.d.
25 °C+90% R.H.		n.d.	n.d.	n.d.
35 °C+80% R.H.		n.d.	n.d.	n.d.

[a] Broader peak at 180–181 °C.
n.d., not detectable.

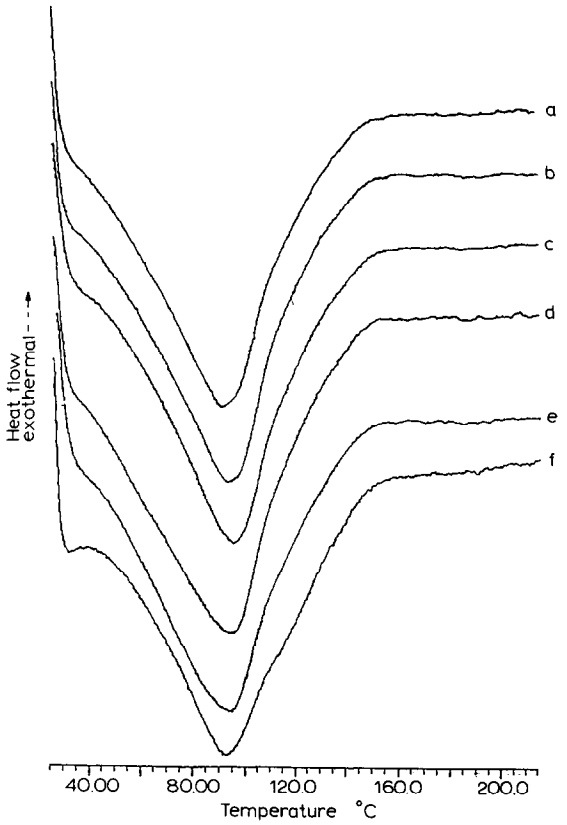

Fig. 4. DSC of FCE24304/β-cyclodextrin 1:2 mol/mol kneaded system: (a) 3 months at 35 °C+80% R.H. storage; (b) 3 months at 25 °C+90% R.H. storage; (c) 3 months at 25 °C+80% R.H. storage; (d) 3 months at 55 °C storage; (e) 3 months at 35 °C storage; (f) initial.

TABLE 2

Percentage of related substances determined by internal normalization of samples stored under different conditions of temperature and humidity as detected by HPLC

	Storage conditions	Initial	1 month	3 months
Parent drug	–	1.00		
	35 °C			1.23
	55 °C		2.23	
1:1 mol/mol system	–	1.05		
	35 °C		0.79	0.96
	55 °C		1.27	6.81
	25 °C + 80% R.H.			0.90
	25 °C + 90% R.H.			0.91
	35 °C + 80% R.H.			0.95
1:2 mol/mol system	–	1.08		
	35 °C			1.02
	55 °C			1.99
	25 °C + 80% R.H.			0.91
	25 °C + 90% R.H.			0.91
	35 °C + 80% R.H.			0.92

Table 3 summarises the observed variations in crystallinity. The differences between the values obtained via DSC vs those estimated from X-rays diffraction are due to the fact that DSC quantifies only the crystallinity of the drug itself, while X-ray analysis evaluates the crystallinity of both drug and β-cyclodextrin.

The dissolution rates of samples stored for 3 months are listed in Table 4 in comparison to the initial values. The 1:2 mol/mol system shows a slight decrease in dissolution rate behaviour which becomes more pronounced for batches exposed to moisture: this may be explained as being due to the partial recrystallization of the complex during storage induced by water uptake. However, such a decrease is not significant for a definitive formulation, the quantity in solution already becoming the same after 10 min.

Similar results were obtained with the 1:1 mol/mol system. Anyway, the dissolution profiles are better for the 1:2 system than for the 1:1 system. For reasons based on both the better dissolution profile and improved stability we have chosen to develop the 1:2 mol/mol system in a tablet formulation.

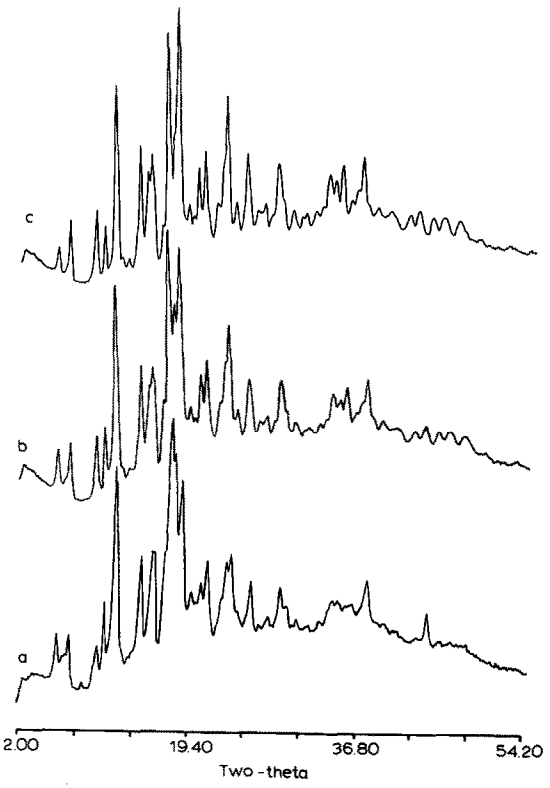

Fig. 5. X-ray diffractograms of FCE24304/β-cyclodextrin 1:1 mol/mol system: (a) initial; (b) 3 months at 35 °C storage; (c) 3 months at 35 °C + 80% R.H. storage.

As a result of the good flowability and compressibility properties of the kneaded complex, the process of direct compression was evaluated, the amount of the other excipients being main-

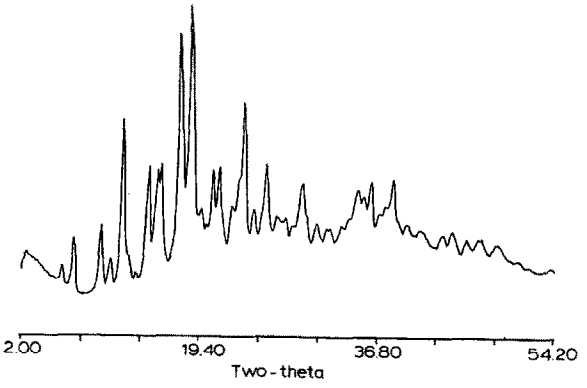

Fig. 6. X-ray diffractogram of FCE24304/β-cyclodextrin 1:2 kneaded system.

TABLE 3

Degree of crystallinity of samples stored under different conditions of temperature and humidity determined by X-ray technique

Storage conditions	Degree of crystallinity (%)			
	Initial	15 days	1 month	3 months
1:1 mol/mol system				
–	60			
35 °C			66	66
55 °C			61	69
25 °C + 80% R.H.		85	89	77
25 °C + 90% R.H.		87	85	82
35 °C + 80% R.H.		81	77	77
1:2 mol/mol system				
–	64			
35 °C				63
55 °C				58
25 °C + 80% R.H.		76	79	76
25 °C + 90% R.H.		81	82	79
35 °C + 80% R.H.		72	73	73

tained as low as possible. Sodium starch glycolate was selected as disintegrant, microcrystalline cellulose as binder/disintegrant and 1% magnesium stearate as lubricant. The tablets prepared had the following properties: weight, 230 mg (RSD 1.2%); diameter, 9 mm (punch concave); thickness, 3.915 ± 0.020 mm; hardness, 4.3 ± 0.6 kp; friability, 0.3%; disaggregation time (USP) 2 min.

Conclusions

On the basis of the data reported above, one may conclude that the technique of preparing a kneaded system is extremely useful for obtaining an FCE24304/β-cyclodextrin complex. The complex exists in the solid state in a 1:2 ratio and is stable with respect to both temperature and humidity.

For the 1:1 system, β-cyclodextrin can mask the degradation of the drug as a consequence of the proportion that can participate in forming the complex. The preliminary data obtained from stability studies performed on FCE24304 tablets demonstrated changes in neither technological nor biopharmaceutical aspects, confirming the results obtained on kneaded products.

TABLE 4

Dissolution rate behavior under sink conditions of samples stored under different conditions of temperature and humidity

Time (min)	Percent in solution					
	Initial	35 °C	55 °C	25 °C + 80% R.H.	25 °C + 90% R.H.	35 °C + 80% R.H.
1:1 mol/mol kneaded system						
1	36	35	36	35	35	35
3	56	54	55	51	54	50
5	68	62	65	60	60	58
10	73	68	74	68	68	67
15	76	72	77	71	71	71
30	80	76	81	77	76	76
60	85	80	84	80	80	80
1:2 mol/mol kneaded system						
1	71	54	56	47	50	49
3	84	75	77	69	71	70
5	88	82	84	76	78	79
10	91	87	89	84	87	85
15	92	89	91	87	88	87
30	95	90	92	89	90	88
60	96	92	95	90	92	90

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