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Note

Separation of tamoxifen geometric isomers and metabolites by bonded-phase β-cyclodextrin chromatography

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Tamoxifen [*trans*-1-(*p*-β-dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene] is a synthetic non-steroidal antiestrogen that is currently widely employed in the chemotherapeutic treatment of estrogen receptor positive human breast cancers and other endocrine tumors. The parent compound is synthesized as both the *cis* and the *trans* isomer, although it is the *trans* isomer that acts as an estrogen antagonist [1]. Several analytical methods, including high-performance liquid chromatography (HPLC), have been described to separate *trans*-tamoxifen

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and its major metabolites that are formed in vivo [2–4]. However, a facile HPLC procedure that can resolve both isomers along with the major plasma metabolites has not been previously reported.

The usefulness of stationary phase cyclodextrins to resolve stereoisomers has recently been described by our laboratory [5]. The present study demonstrates the application of β-cyclodextrin HPLC for the separation of *cis*- and *trans*-tamoxifen, as well as for the separation of the major plasma metabolites of *trans*-tamoxifen, 4-hydroxytamoxifen and N-desmethyltamoxifen. In addition, computer modeling of the X-ray crystal structure inclusion complex is shown, demonstrating why the *cis*- and *trans*-tamoxifen are resolved by β-cyclodextrin.

EXPERIMENTAL

The *cis* and *trans* forms of tamoxifen and 4-hydroxy- and N-desmethyltamoxifen were all provided by Imperial Chemical Industries (Chesire, U.K.). HPLC separation was completed using a Shimadzu LC-4A liquid chromatograph equipped with a variable-wavelength detector set at 254 nm. The β-cyclodextrin columns, packed at 13 000 p.s.i. maximum pressure with a Haskell pump, were purchased from Advanced Separation Technologies (Whippany, NJ, U.S.A.). For the isolation of tamoxifen metabolites from human plasma, 1 ml of plasma was extracted with 10 ml of hexane containing 2% butanol, as has been described elsewhere [2]. Separations were completed using either a 10 cm×2.2 cm β-cyclodextrin column for the separation of *cis*- and *trans*-tamoxifen or 25 cm×2.2 cm columns for the separation of the *trans*-tamoxifen metabolites. The respective mobile phase conditions are described under the results section.

RESULTS AND DISCUSSION

Separation of cis- and trans-tamoxifen

The resolution of *cis*- and *trans*-tamoxifen was easily obtained using a 10 cm×0.46 cm β-cyclodextrin HPLC column with a mobile phase consisting of methanol–water (50:50). Under conditions of a flow-rate of 1 ml/min at room temperature, the retention times for *cis*- and *trans*-tamoxifen were 3.1 and 6.2 min, respectively, as is shown in Fig. 1. Peak resolution was very good with a resolution factor and separation factor of 1.91 and 2.34, respectively. In order to understand the basis for the separation of these geometric isomers by β-cyclodextrin, we examined the respective interaction of the crystal structures of *cis*- and *trans*-tamoxifen with β-cyclodextrin using computer graphic modeling. These interactions are shown in Fig. 2, which were obtained using Van der Waal's surface (shown as dots) to model the inclusion complex of *cis*- or *trans*-tamoxifen with β-cyclodextrin. It is apparent that *trans*-tamoxifen is able to form a better inclusion complex than *cis*-tamoxifen. The phenyl side-chain of the *trans* isomer was found to penetrate deeper in the β-cyclodextrin cavity compared to the *cis* isomer, as can be seen in Fig. 2. This difference was quantified by measuring the distance from the mouth of the β-cyclodextrin (at the 2-hydroxyl atom) to the deepest penetrating atom of the respective tamoxifen species. Using the crystal

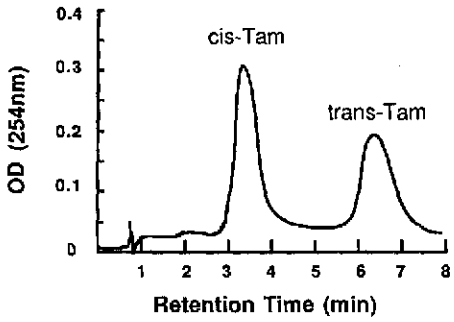

Fig. 1. Chromatographic separation of *cis*-tamoxifen (cis-Tam) and *trans*-tamoxifen (trans-Tam). A sample containing 0.3 μg *cis*- and *trans*-tamoxifen was loaded onto a 10 cm × 0.46 cm β-cyclodextrin column, with the separation completed at a flow-rate of 1 ml/min isocratically using a mobile phase of methanol–water (50:50).

structure model shown, the average distance from the 3-hydroxyl of the β-cyclodextrin to the lowest atom of the respective tamoxifen isomer was measured. The *trans* isomer entered the cavity at a distance of 6.3 Å, while the *cis* isomer penetrated only 5.7 Å. The difference in penetration allows for *trans*-tamoxifen to be more tightly complexed in the hydrophobic cavity of the β-cyclodextrin, as well as for additional interaction to result between the side-groups of the *trans* isomer and the outer 2′- and 3′-hydroxyl atoms of the β-cyclodextrin. This form of tamoxifen is thereby retained longer on the column.

These studies demonstrate the useful predictive power that computer modeling has for predetermining the ability of β-cyclodextrin chromatography to resolve geometric isomers, in addition to allowing for the understanding of the structural requirements needed for resolution to occur. This utility has been suggested for optical isomers in a previous study by our laboratories [5].

Separation of tamoxifen plasma metabolites

Human plasma was spiked with varying amounts of *cis*-, *trans*-, 4-hydroxy- and N-desmethyltamoxifen and then extracted as described in Experimental. Following solubilization in methanol–water (50:50), a 100 μl sample was injected onto two 25 cm × 0.46 cm β-cyclodextrin columns connected in series. Elution was carried out at 1 ml/min using a 20-min gradient from 10 to 25% acetonitrile containing 1% triethylammonium acetate (pH 4). These conditions allowed for resolution of each of these compounds with retention times (Table I) of 31.6, 38.5, 40.0 and 43.5 min for *cis*-, *trans*-, N-desmethyl- and 4-hydroxytamoxifen, respectively. This extraction and analysis procedure allows for excellent recovery and quantitation and is therefore useful for the study of tamoxifen metabolism and pharmacokinetics. It should be noted, however, that for evaluation of clinical serum samples of patients receiving tamoxifen, photoactivation of the column effluent is desired to enable submicromolar concentrations of these compounds to be monitored by fluorescence activation (please refer to ref. 2 and 3 for more complete descriptions of this procedure).

These results demonstrate the application of bonded-phase β-cyclodextrin

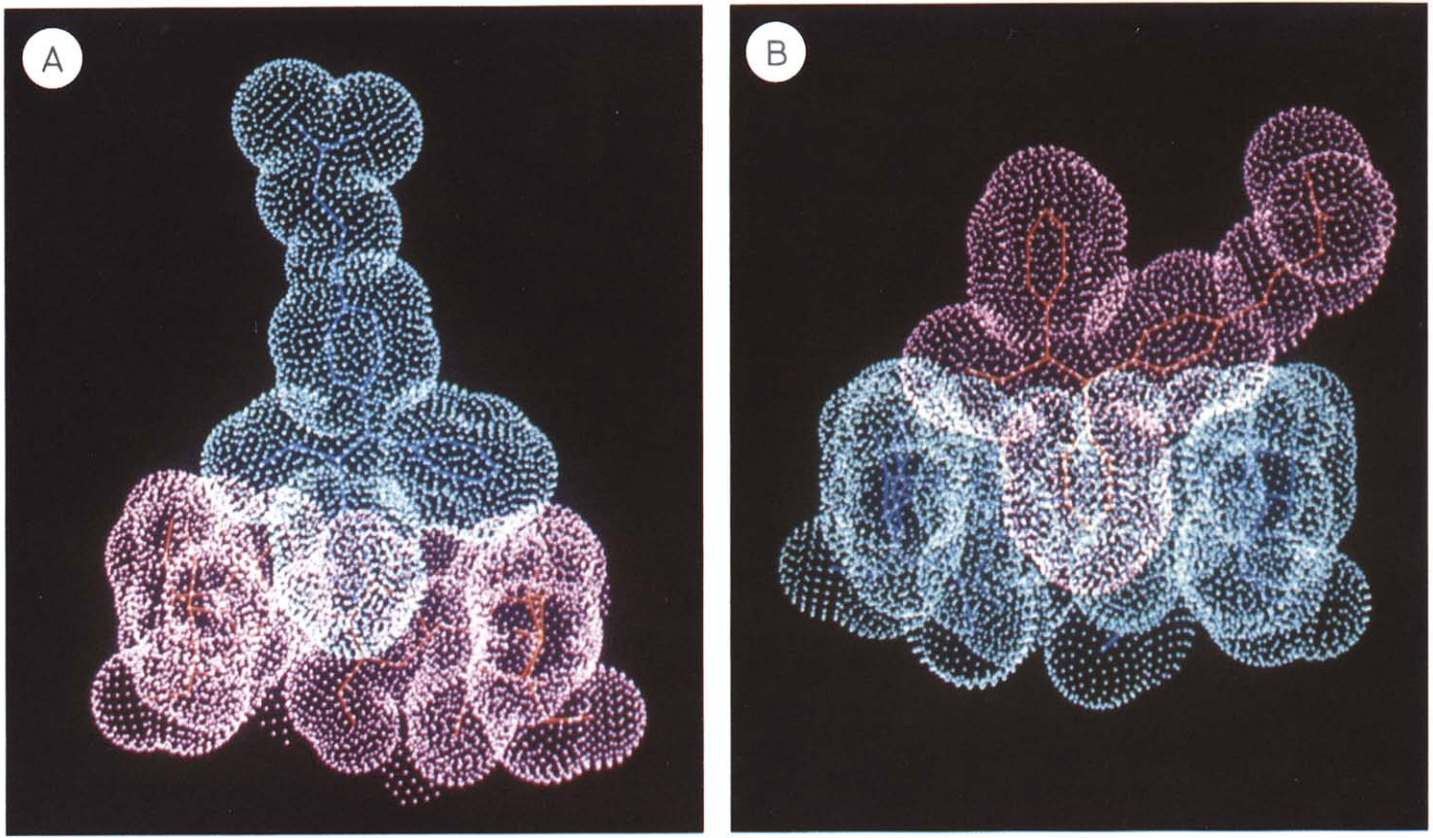

Fig. 2. The inclusion complex formed between β-cyclodextrin and *cis*-tamoxifen (A) or *trans*-tamoxifen (B). The X-ray crystal structure of each molecule was projected using computer graphics, and the optimal inclusion complex for each geometric isomer of tamoxifen determined using the Van der Waal's surface interaction (shown by the dots) for modeling.

TABLE I

CHROMATOGRAPHIC RESOLUTION OF TAMOXIFEN GEOMETRIC ISOMERS AND METABOLITES

Human plasma was spiked with *cis*-, *trans*, 4-hydroxy- and N-desmethyltamoxifen, and then extracted as described in Experimental. The extract was then separated by HPLC analysis using β-cyclodextrin columns.

Compound	Capacity factor	Separation factor	Resolution factor
cis-Tamoxifen	4.45		
		1.19	6.83
trans-Tamoxifen	5.64		
		1.05	1.0
N-Desmethyltamoxifen	5.90		
		1.10	1.98
4-Hydroxytamoxifen	6.50		

chromatography for the separation of the tamoxifen geometric isomers, as well as the major metabolites of the antiestrogen *trans*-tamoxifen. Although not attempted, the resolution of the plasma metabolites of *cis*-tamoxifen should also be easily obtainable using this system.

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