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Short Communication

Analysis of tamoxifen isomers in tablet preparations by high-performance liquid chromatography

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Introduction

Tamoxifen citrate is a non-steroidal anti-oestrogenic compound used in the treatment of breast cancer and anovulatory infertility It occurs as two geometric isomers only the *Z* (*trans*) of which is active A variety of methods of analysis have been used for the determination of tamoxifen in biological fluids including high-performance liquid chromatography (HPLC) [1–9], thin-layer chromatography [10], and gas chromatography [11] Two of these publications have reported a resolution of both isomers [6, 7] Methods have also been described [12–14] for the analysis of tamoxifen in its dosage form but only one method [14] has been concerned with quantifying the two isomers, that method was recommended as suitable for determining the *E* (*cis*)-isomer content of the drug in dosage forms

An *E*-isomer limit test for tamoxifen citrate forms part of the monographs of the British Pharmacopoeia (BP 1988) and United States Pharmacopeia (XXI) The BP 1988 also includes a limit test for the *E*-isomer content of the tablets, using reversed-phase HPLC This method has been used in this laboratory, but a high eluent pH and poor resolution have presented difficulties The assay for total tamoxifen citrate in the tablet monograph involves the ultraviolet absorbance measurement of methanolic extracts The present work proposes an extension of this assay by separation and simultaneous quantitation of both *E*- and *Z*-isomers using reversed-phase ion-pairing chromatography

Experimental

Reagents and chemicals

Methanol (Analar grade) and *ortho*phosphoric acid (general purpose reagent), were

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supplied by BDH Ltd, acetonitrile (HPLC grade) by Rathburn Chemicals Ltd, heptane sulphonic acid (0 1 M) by FSA Laboratory Supplies, and tetraethylammonium bromide by Aldrich Chemicals Co

Chromatography

HPLC was carried out on a Spectra Physics (Hemel Hempstead, England) model 8100 liquid chromatograph connected to a 8440 variable wavelength UV detector and 4100 computing integrator The autosampler was fitted with a 40-μl injection loop The eluent was acetonitrile–aqueous phase (70 30, v/v), the aqueous phase was prepared by adding 50 0 ml of 0 1 M heptane sulphonic acid and 2 625 g of tetraethylammonium bromide to sufficient water to produce 500 ml The pH of the aqueous phase was adjusted to 2 3 with *ortho*phosphoric acid The eluent was pumped through a stainless steel column (250 × 4 6 mm i d) packed with Spherisorb 5 ODS (Phase Separations Ltd, Deeside, Wales), at a flow rate of 1 2 ml min^{-1} Peak responses were measured by area integration, for quantification of total tamoxifen, the sum of the peak areas for each isomer was used

Standard preparation

Reference tamoxifen base *E*-isomer (99 9% pure) and reference tamoxifen citrate *Z*-isomer (99 5% pure) were kindly provided by Leiras Medica (Turku, Finland) Both were used as received The working HPLC standard was tamoxifen citrate BPCRS (British Pharmacopoeia Commission, batch 1166) with a declared *E*-isomer content of 1 2%, m/m The working HPLC standard solutions were prepared by dissolving 20 mg of the standard into sufficient methanol to produce 100 0 ml Aliquots were transferred into autosampler vials made of amber glass Standard solutions for assessment of linearity were prepared separately by adding 5–25 mg of reference tamoxifen citrate *Z*-isomer into sufficient methanol to produce 100 0 ml, and 0 05 to 1 25 mg of reference tamoxifen base *E*-isomer in sufficient methanol to produce 100 0 ml Solutions were transferred to autosampler vials made of amber glass

Sample preparation

The samples were prepared by weighing and crushing 10 tablets and adding a quantity of powder equivalent to 20 mg of tamoxifen into a 100-ml amber volumetric flask Methanol (50 ml) was added and the flask shaken for 1 min with periodic ultrasonication The solution was diluted to 100 0 ml, mixed, and a portion centrifuged at 4500 rpm for 2 min An aliquot was transferred into an autosampler vial made of amber glass All standard and sample solutions were protected from light

Results and Discussion

A good separation of *E*- and *Z*-isomers was achieved (resolution = 1 7) with respective capacity factors of 3 7 and 4 2 (Fig 1) The linearity of response was confirmed separately for each isomer The coefficients of linear regression (r) were 0 9997 for the *Z*-isomer and 0 9974 for the *E*-isomer The precision (as relative standard deviation) for repeated injections (n = 8) of the HPLC working standard solution was 0 5% for the *Z*-isomer peak and 1 1% for the *E*-isomer peak

For total tamoxifen determination a comparison of the BP (1988) method and the proposed HPLC method was made on a batch of film-coated tablets (Evans Medical Ltd, Speke, Liverpool) Results of 20 26 and 20 0 mg/tablet were obtained for the BP method

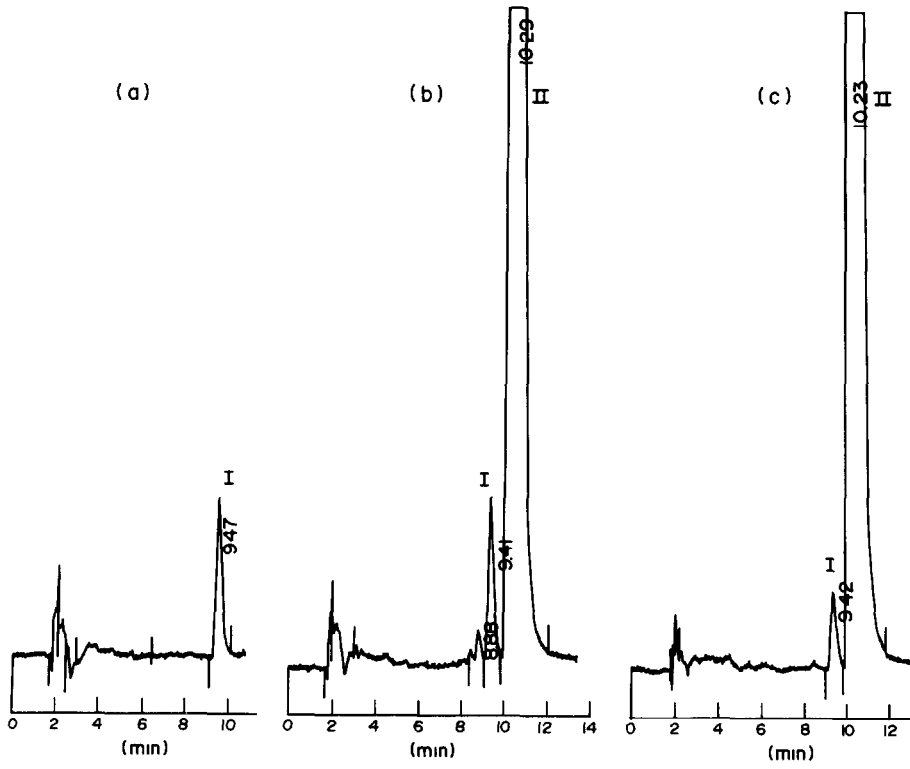

Figure 1
Representative chromatograms obtained for (a) reference tamoxifen *E*-isomer 0 15 mg/100 ml in methanol, (b) the HPLC working standard tamoxifen citrate BPCRS 20 mg/100 ml in methanol and (c) a processed sample of tamoxifen tablets I refers to the *E*-isomer and II to the *Z*-isomer of tamoxifen

and the HPLC method, respectively The *E*-isomer content measured by the HPLC method was 0 11 mg/tablet It was not possible to use the BP (1988) HPLC method for the *E*-isomer measurement and so the comparison was not made However, an assay of the working HPLC standard using the reference tamoxifen *E*-isomer gave a result of 1 23% m/m

The repeatability of the assay was determined by repeatedly assaying the same batch of film-coated tablets Results of 1 1% relative standard deviation (x = 20 0 mg/tablet, n = 6) for total tamoxifen and 2 4% relative standard deviation (x = 0 11 mg/tablet, n = 6) for the *E*-isomer were obtained

The method described is a reliable and simple assay for the simultaneous quantification of the *Z*- and *E*-isomers of tamoxifen Its application to tablet preparations has been demonstrated but could be adopted as the test for *E*-isomer content in raw material samples of tamoxifen

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