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High-performance liquid chromatographic analysis of FCE 24304 (6-methylenandrosta-1,4-diene-3,17-dione) and FCE 24928 (4-aminoandrosta-1,4,6-triene-3,17-dione), two new aromatase inhibitors

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ABSTRACT

The cytochrome P-450-dependent aromatase enzyme plays an important role in hormone-dependent diseases. Many products that inhibit this type of enzyme were obtained: FCE 24304 (I) and FCE 24928 (II) proved to possess remarkable activity and are presently under development. Compounds I and II and their synthetic intermediates are analyzed by means of a high-performance liquid chromatographic method, affording rapid and efficient separation, good resolution and identification of all the examined compounds. The linearity, specificity, sensitivity, precision and accuracy for the method are also provided.

INTRODUCTION

Aromatase, a cytochrome P-450-dependent enzyme, catalyzes the conversion of androgens to estrogens [1]. This enzyme affects the rate-limiting step in estrogen production and therefore it is supposed to play a key role in the pathogenesis of estrogen-dependent diseases.

The conversion of androgens to estrogens by aromatase has been demonstrated [1–4], the mechanism of aromatization reported [5], and the enzyme purified from human placental microsomes [5–9].

A series of steroid compounds were synthesized and tested for their aromatase inhibitory activity. Among them FCE 24304 (I, 6-methylenandrosta-1,4-diene-3,17-dione) and FCE 24928 (II, 4-aminoandrosta-1, 4, 6-triene-3,17-dione) (Fig. 1) exhibited good activity and remarkable stability [10,11].

In this paper a high-performance liquid chromatographic (HPLC) method for quantitation of FCE 24304 (I) and FCE 24928 (II) and separation of related synthetic intermediates is reported, and the experimental parameters for separation of artificial mixtures of I, II, and related substances are described (Figs. 2 and 3).

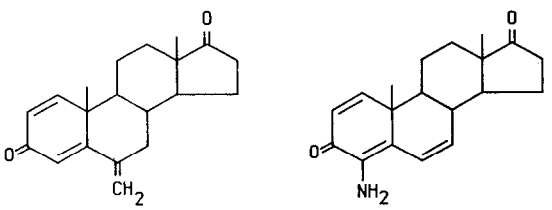

Fig. 1. Chemical structures of FCE 24304 (I) and FCE 24928 (II).

EXPERIMENTAL

HPLC-grade acetonitrile and other chemicals of analytical grade were obtained from Carlo Erba (Milan, Italy). Buffer solutions were filtered before use through a Millipore 0.45-μm filter.

The HPLC system consisted of a Hewlett-Packard (HP) 1090 LUSI chromatograph equipped with

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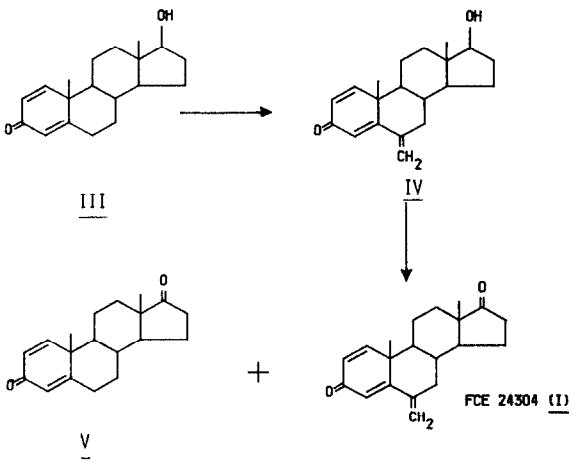

Fig. 2. Synthesis of FCE 24304 (I).

an HP 79847B temperature-controlled autosampler, and HP 1040A variable-wavelength detector (with a DPU multichannel integrator and an HP 85 B computer), an HP 2225 think-jet printer and an HP 7470A plotter.

Phosphate buffer (eluent A) was prepared by dissolving dibasic ammonium phosphate (6.60 g, 0.05 mol) in deionized water (1000 ml) filtered with a Milli-Q3 system (Waters), and adjusting to pH 6.0 with concentrated phosphoric acid; eluent B consisted of a mixture of eluent A and acetonitrile (30:70, v/v).

Isocratic elution with 45% B for 3 min followed by a linear gradient to 96% B in 20 min was used.

A Partisphere $5C_{18}$ cartridge (Whatman, 110 × 4.7 mm I.D., 5 μm particle size), at 40°C with flow-rate of 1 ml/min was used.

UV detection was at 245 and 220 nm. The samples were dissolved in eluent A–acetonitrile (90:10, v/v) immediately before use.

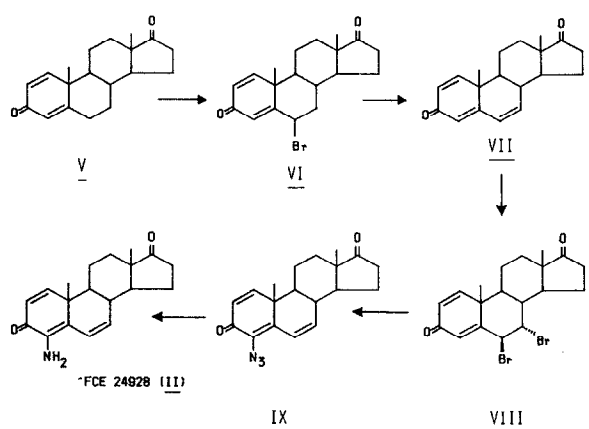

Fig. 3. Synthesis of FCE 24928 (II).

RESULTS AND DISCUSSION

The described gradient elution, with increasing acetonitrile content, is necessary for selective elution of the synthetic intermediates.

The reproducibility of the chromatographic system is high: the resolution of the compounds of interest does not change whereas, as expected, the relative retention times decrease with increasing column lifetime. The retention times of the compounds change by no more than approximately 1% during an 8-h run; however, it is possible to use the column for several months of continuous use.

Two chromatograms of an artificial mixture of compounds I and III–V and compounds II and V–IX are shown in Fig. 4; all the products were dissolved in eluent A (ammonium phosphate 0.05 *M*, pH 6.0) containing a small amount of acetonitrile (about 10%).

Note that the by-product (V) in the synthesis of FCE 24304 (I) is also the starting material for the synthesis of FCE 24928 (II) (see Figs. 2 and 3).

The elution patterns, with increasing retention times, are in the order: III, V, IV, I and II, VII, V, VI, VIII, IX. Other impurities are also well separated. The chromatographic method provides good resolution of all peaks and allows separation, identification and quantitative determination of these compounds, if present.

Satisfactory results for FCE 24304 (I) and FCE 24928 (II), in terms of both linearity and of sensitivity [quantitation limits are better than 0.5 μg/ml (I) and 0.75 μg/ml (II)] were obtained; linear relationships between peak areas and amounts of products injected are observed in the ranges 10–90 μg/ml (I) and 60–490 μg/ml (II).

Regression equations

Area = slope × concentration + intercept.

(I) Area counts = 109.4 × (concentration in μg/ml) + 15.6

(II) Area counts = 33.1 × (concentration in μg/ml) + 227.7

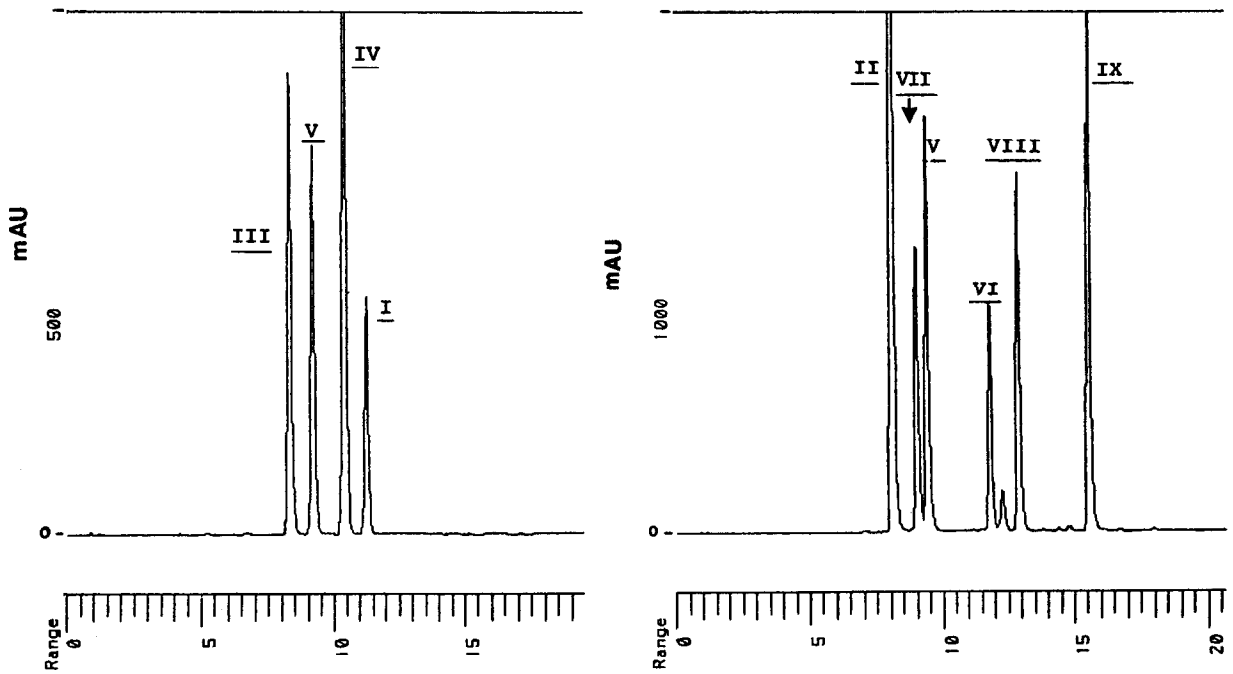

Fig. 4. Chromatograms of artificial mixtures of products I and III–V and products II and V–IX; conditions as reported in the text.

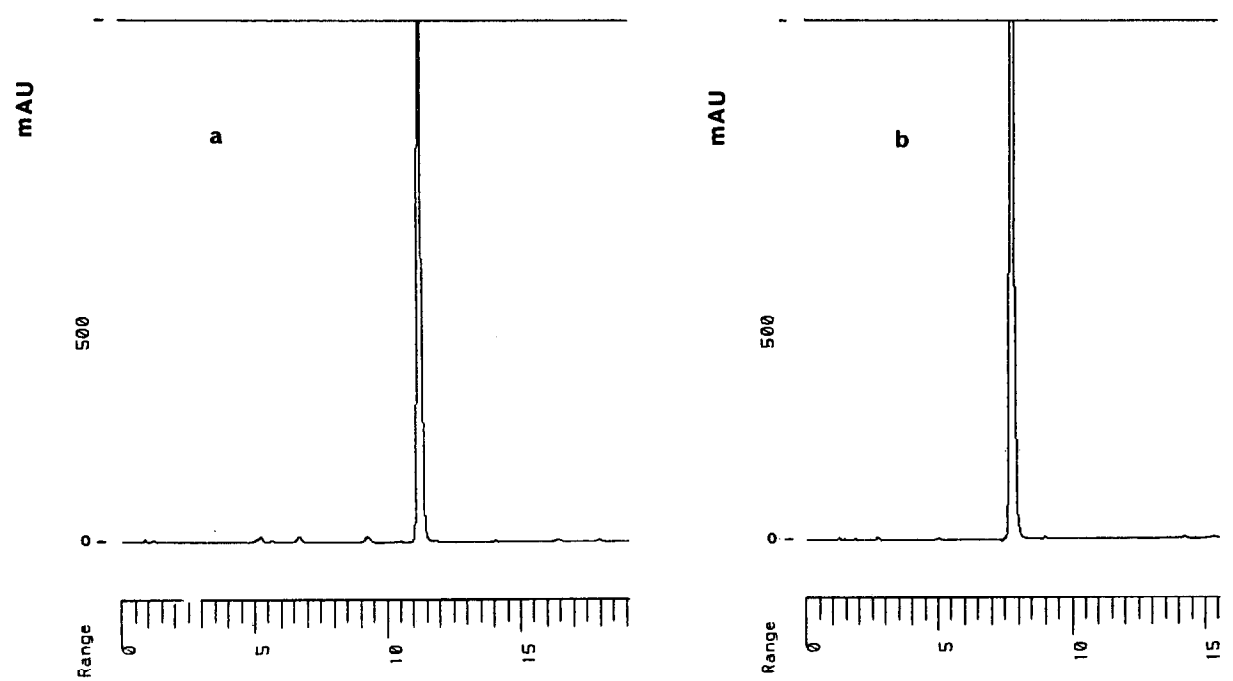

Fig. 5. Chromatograms of representative lots of I (a) and II (b).

From calibration plots, the relative correlation coefficients are: FCE 24304 (I), $r = 0.99968$; FCE 24928 (II), $r = 0.99753$.

The chromatographic method provides also satisfactory specificity, precision and accuracy for I and II analyses.

Specificity

No interferences of sample solvent [0.05 *M* ammonium phosphate, pH 6.0–acetronitrile (90:10, v/v)] and synthetic impurites (see Figs. 2 and 3) could be observed at the detection wavelength (245 nm), as shown in Fig. 5.

From the chromatograms, only product (V) is recognized as an impurity in FCE 24304 (I), but it was not determined quantitatively. However, the separation of very low amounts of unknown impurities is also observed.

Precision

The following results were obtained from seven replicate injections of the same solutions. FCE 24304 (I): concentration = 105.6 μg/ml; S.D. = ± 0.389; precision = ± 0.95%. FCE 24928 (II): concentration = 220.2 μg/ml; S.D. = ± 0.248; precision = ± 0.60%.

Accuracy

From three replicate injections of every solution at five different concentrations the accuracy was for FCE 24304 (I), S.D. = ± 0.761; for FCE 24928 (II), S.D. = ± 0.540.

Stability

The stabilities of I and II in the injection solution were also studies with the present chromatographic system. The solutions (at concentrations of about 100 and 200 μg/ml, respectively) were stored at room temperature (about 25°C) and the area counts of the relative peaks with respect to the initial time were measured as function of the time.

Under these conditions FCE 24304 (I) proved to be stable, whereas FCE 24928 (II) slowly decomposed with formation of one degradation product (with a shorter retention time than that of FCE 24928), at present unidentified.

CONCLUSIONS

The use of the described HPLC method allows a selective and quantitatively accurate analysis of steroid aromatase inhibitor compounds and the determination of possible impurities in the active drug substance. The chromatographic method is sufficiently specific, accurate, precise and sensitive for the purpose of analytical characterization.

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