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Note

Determination of etozolin and ozolinone in human plasma and tissues by reversed-phase high-performance liquid chromatography

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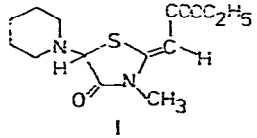
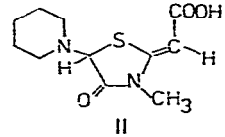
Etozolin [ethyl(*Z*)-(3-methyl-4-oxo-5-piperidino-thiazolidin-2-ylidene)acetate (I) is a novel diuretic drug that is rapidly metabolized by hydrolysis of the ester group to give the free acid ozolinone, *Z*-(3-methyl-4-oxo-5-piperidino-thiazolidin-2-ylidene)acetic acid (II). Ozolinone itself exhibits a high pharmacological activity and has a longer half-life in man than etozolin, so that an estimation of the concentration of both I and II is of interest in pharmacological investigations [1]. A thin-layer chromatographic (TLC) method [2,3] and a high-performance liquid chromatographic (HPLC) method [4] for the estimation of I and II have been reported. The HPLC method was reported to be more sensitive and accurate and less prone to interference than the TLC method [4].



I



II

In the HPLC method, adsorption chromatography on a LiChrosorb Si-100 column using cyclohexane—chloroform mixtures for elution was described. Because of the cost of the elution mixtures, the toxicity of chloroform and the limited life-span of the column used, we have chosen to develop a reversed-phase HPLC method.

EXPERIMENTAL

Compounds I and II, ethyl(*Z*)-(3-ethyl-4-oxo-5-piperidino-thiazoldin-2-ylidene) acetate (internal standard I), and (*Z*)-(3-ethyl-4-oxo-5-piperidino-thiazolidine-2-ylidene)acetic acid (internal standard II), were generously donated by Gödecke, Freiburg, G.F.R. Methanol was LiChrosolv, other chemicals were of analytical grade and obtained from E. Merck (Darmstadt, G.F.R.).

HPLC measurements were made using a Perkin-Elmer Model 2/2 liquid chromatograph equipped with a Model LC 75 UV detector and a Rheodyne 7105 valve. Stainless-steel columns (25 cm × 4 mm, Knauer, Berlin, G.F.R.) were packed with LiChrosorb RP-18, 7 μm (Merck) and fitted with a pre-column (4 cm × 4 mm) filled with the same material.

Plasma (1—2 ml) was selectively extracted with dichloromethane according to the method of Hengy et al. [4], to give two extracts for each plasma sample. Extract I was obtained from alkalinized plasma and contained I and internal standard I. Extract II was obtained from reacidified plasma and contained II and internal standard II.

Tissue was homogenized with four times its own weight of triple-distilled water using a Polytron homogenizer. Five millilitres of homogenate were then extracted in a similar manner to plasma.

Aliquots of 3—10 μl of extract I were injected on to the HPLC column and eluted isocratically with 65% methanol in 20 m*M* phosphate buffer at pH 2.2 (Fig. 1). Aliquots of 3—10 μl of extract II were injected on to the HPLC column and eluted isocratically with 40% methanol in 20 m*M* phosphate buffer at pH 2.2 (Fig. 2). Calibration curves (Fig. 3) were prepared each day by adding I and II to drug-free plasma or tissue homogenates and handling these in the manner outlined above. Peak height ratios were used in all determinations. The UV detector was operated at 282 nm for the analysis of both I and II.

RESULTS AND DISCUSSION

Although I and II may be analyzed by reversed-phase HPLC in one run using gradient elution, such a procedure could not be used for the assay of low drug concentrations in tissues (below 100 ng/g) due to noisy baselines and interfering peaks. By utilizing the selective extraction of I and II and their respective internal standards and analyzing for I and II separately using reversed-phase HPLC and isocratic elution, the sensitivity of the assay could be greatly increased and, even more importantly, such procedures were applicable to the analysis of low drug levels in tissues. Strictly linear calibration curves (Fig. 3) were obtained for the estimation of I and II in the concentration ranges from 30 ng/ml to 10 μg/ml. The lower limit of detection for both I and II was approximately 10 ng/ml. The regression coefficients of the calibration curves always exceeded 0.99 for both I and II. The relative standard deviation of the method, as determined by analysis of multiple serum samples containing 1 μg/ml, was between 4 and 6% for both I and II.

The use of pre-column, which was normally changed after approximately 200 injections, effectively protected the column from damage. A column in everyday use had an average lifetime of more than 4 months.

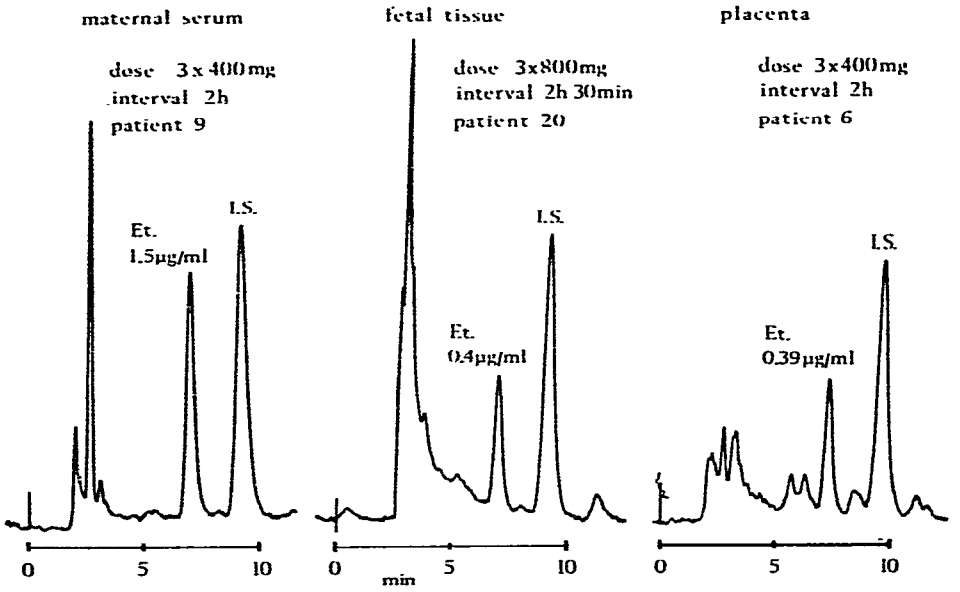


Fig. 1. Analysis of etozolin (Et.) in human serum, placenta and fetal tissue. Solvent: 65% methanol in 20 m*M* phosphate buffer (pH 2.2). I.S. = internal standard I; interval = time between the dose and interruption of pregnancy by curettage (first trimester).

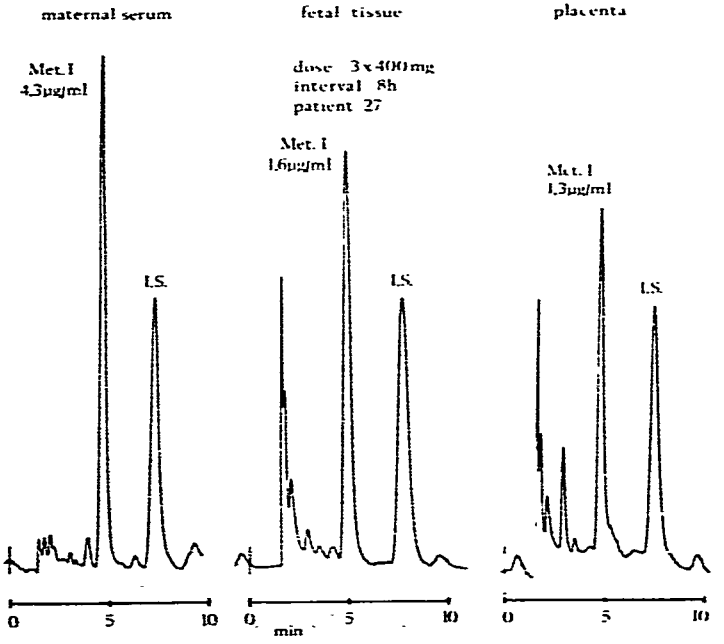


Fig. 2. Analysis of ozolinone (Met. I) in human serum, placenta and fetal tissue. Solvent: 40% methanol in 20 m*M* phosphate buffer (ph 2.2). I.S. = internal standard II; interval = time between the dose and interruption of pregnancy by curettage (first trimester).

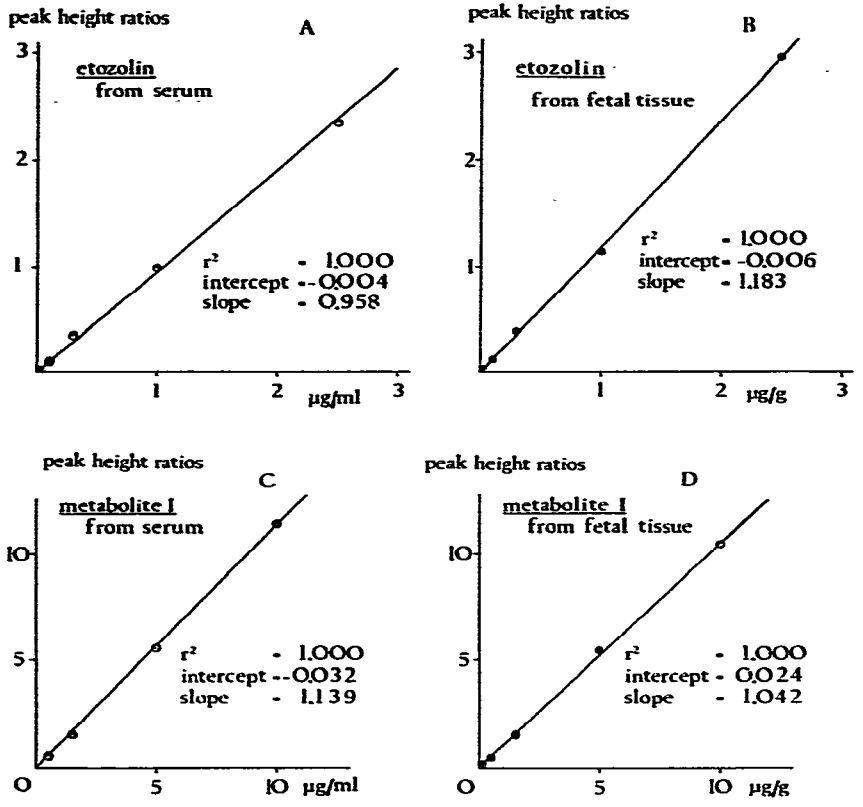


Fig. 3. Plots of peak height ratios (A and B: etozolin/I.S. I; C and D: ozolinone/I.S. II) versus amounts of etozolin and ozolinone added to serum (A and C) and tissue homogenates (B and D).

In spite of the simplicity of the assay procedures developed, I and II could be determined in small samples of blood and, without modification, also in tissue homogenates following therapeutic doses of etozolin (Figs. 1 and 2). Low-cost columns (see Experimental) with theoretical plate numbers of 8000—12,000/m are sufficient for all analytical problems encountered so far.

The method described here has been in continual use in our laboratory for more than one year for the investigation of the placental transfer of I and II during the first and second trimester of human pregnancy. Both I and II were found in fetal and placental tissue in lower concentrations than in maternal serum (fetal tissue/maternal serum ratios of between 0.3 and 0.6). Also, the drugs were distributed rather evenly within the human fetal compartment. Drug concentrations in the blood of pregnant patients were lower than in non-pregnant patients (unpublished results).

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