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QUANTITATIVE ANALYSIS OF OXEPINAC IN HUMAN PLASMA, URINE AND SALIVA BY GAS CHROMATOGRAPHY—MASS FRAGMENTOGRAPHY

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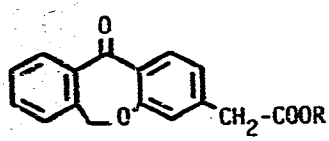
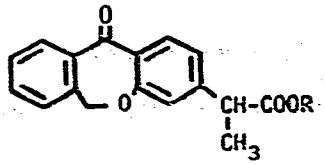
SUMMARY

A sensitive and specific method is described for the quantitative analysis of 6,11-dihydro-11-oxo-dibenz[*b*,*e*]oxepin-3-acetic acid (oxepinac) in human plasma, urine and saliva. Oxepinac and internal standard are extracted from acidified plasma, urine or saliva, converted to the corresponding *n*-propyl esters and analysed by gas chromatography—mass fragmentography using selected ion monitoring. The method is accurate and precise over the range 100 μg/ml to 1.0 ng/ml. The method has been applied to the analysis of plasma, urine and saliva from healthy volunteers receiving therapeutic doses of oxepinac.

INTRODUCTION

The compound 6,11-dihydro-11-oxo-dibenz[*b*,*e*]oxepin-3-acetic acid, oxepinac (also DD-3314; I in Fig. 1), is currently being developed as a new anti-inflammatory agent [1, 2]. The determination of plasma levels and urinary excretion of the drug in man has become more important, because they afford valuable information concerning the bioavailability of the drug and its therapeutic and toxic thresholds. It was expected, from the known metabolism of oxepinac in animals [3], that the plasma levels in man would be in the range 0.4—1.5 μg/ml following an initial oral dose of 12.5 mg of oxepinac. Thus a sensitive and specific analytical method for oxepinac in plasma and urine had to be developed. After conversion of oxepinac to its *n*-propyl ester (II in Fig. 1), satisfactory separation by gas chromatography and sufficient sensitivity was available from detection with selected ion monitoring in gas chromatography—mass fragmentography. Saliva is a more convenient fluid to obtain than blood for studies on drug disposition. In order to study the correlation between plasma and salivary concentrations, we attempted to determine the saliva





I R = H

II R = CH_3-CH_2-CH_2-

III = H

IV = CH_3-CH_2-CH_2-

Fig. 1. Structural formulae of oxepinac (I) and DD-3505 (III), and of their *n*-propyl esters (II and IV, respectively).

levels of oxepinac after an oral dose in man. Saliva levels in man could also be detected by this analytical method.

EXPERIMENTAL

Material and reagents

All solvents and reagents were of analytical reagent grade and were used without further purification. Oxepinac (I) and DD-3505 (III) were synthesized in the Research Institute of Daiichi Seiyaku Co.

Gas chromatography—mass fragmentography

An Hitachi Model RMU-6MG mass spectrometer equipped with a gas chromatograph was used. The gas chromatographic conditions for oxepinac *n*-propyl ester (II) were as follows: a glass column (1 m × 3 mm I.D.) containing 2% OV-17 on gas-Chrom Q (80—100 mesh); the temperatures of the oven, the injection port and the separator were 270°, 310° and 310°, respectively. The carrier gas (helium) flow-rate was 30 ml/min in all instances. Mass spectrometric conditions in all instances were as follows: ionization voltage, 30 eV; target current, 100 μA; ion source temperature, 200°; multiplier potential, 2 kV. For selected ion monitoring, a multiple ion detector was employed. The following ion *m/e* focusing was used: *m/e* 310 for oxepinac *n*-propyl ester (II) and *m/e* 324 for DD-3305 *n*-propyl ester (IV).

Analytical procedure

To 1 ml of plasma, 0.1 ml of the internal standard solution (corresponding to 5 μg of DD-3305) and 0.5 ml of 1 *N* hydrochloric acid were added. The solution was extracted with exactly 5 ml of isopropyl ether by shaking for 15 min on an automatic shaker. After centrifugation, exactly 4.5 ml of the organic phase were transferred to a glass tube. The solvent was heated to 40° in a water-bath and removed by a gentle stream of nitrogen. The residue was dissolved in 1 ml of *n*-propanol saturated with hydrogen chloride. The mixed solution was heated at 60° for 1 h. After the reaction, *n*-propanol was evaporated under reduced pressure. To the residue, 1 ml of 3% sodium bicarbonate solution and 5 ml of chloroform were added. The tube was carefully shaken for 2 min. The organic phase, after being transferred to a test-tube, was heated to 40° in a water-bath and removed by a gentle stream of nitrogen.

The residue was then dissolved in 200 μl of *n*-propanol. A volume of 1 or 2 μl of this solution was injected into the gas chromatographic colum of the gas chromatography—mass fragmentography system.

To 1 ml of urine sample, stored at −20°, 0.1 ml of the internal standard solution (corresponding to 20 μg of DD-3505) and 0.5 ml of 1 *N* hydrochloric acid were added and extracted with 5 ml of isopropyl ether; the extract was processed in the same manner as described for the plasma sample.

For the analysis of the conjugate in urine, 1 ml of 2 *N* sodium hydroxide and 0.1 ml of internal standard solution were added to 1 ml of urine. The mixture was heated at 80° for 1 h. After cooling, 2 ml of 4 *N* hydrochloric acid were added, and the mixture subsequently processed in the same manner as described above.

To 2 ml of saliva, 0.1 ml of the internal standard solution (corresponding to 0.5 μg of DD-3505) and 0.5 ml of 1 *N* hydrochloric acid were added, and then processed in the same manner as described for plasma samples.

RESULTS AND DISCUSSION

Internal standard

DD-3505, 2-(6,11-dihydro-11-oxodibenz[*b*,*e*]oxepin-3-yl)propionic acid (III) was used for internal standard (I.S.). On the gas chromatography—mass spectrogram of the *n*-propyl ester of I.S. (IV), the fragment ion *m/e* 310, which was the selected monitoring ion for analysis of the *n*-propyl ester of oxepinac (II), was almost not detected (Figs. 2 and 3). The *n*-propyl esters of oxepinac (II) and I.S. (IV) had almost the same retention times under the gas chromatographic conditions used.

Conjugates

After the administration of oxepinac, large amounts of conjugated metabolites (mainly the acyl glucuronide of oxepinac) are excreted in the urine. The acyl glucuronide of oxepinac was completely converted into free oxepinac by alkaline hydrolysis. When the urinary conjugate was to be analyzed, the urine was first treated with alkali and then the total oxepinac, comprising free and conjugated oxepinac, was analysed. The acyl glucuronide decomposed easily

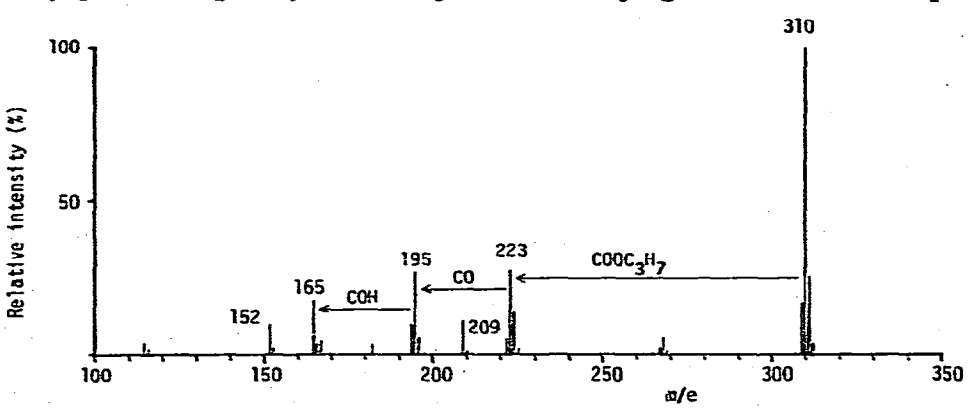


Fig. 2. Mass spectrum of oxepinac *n*-propyl ester (II).

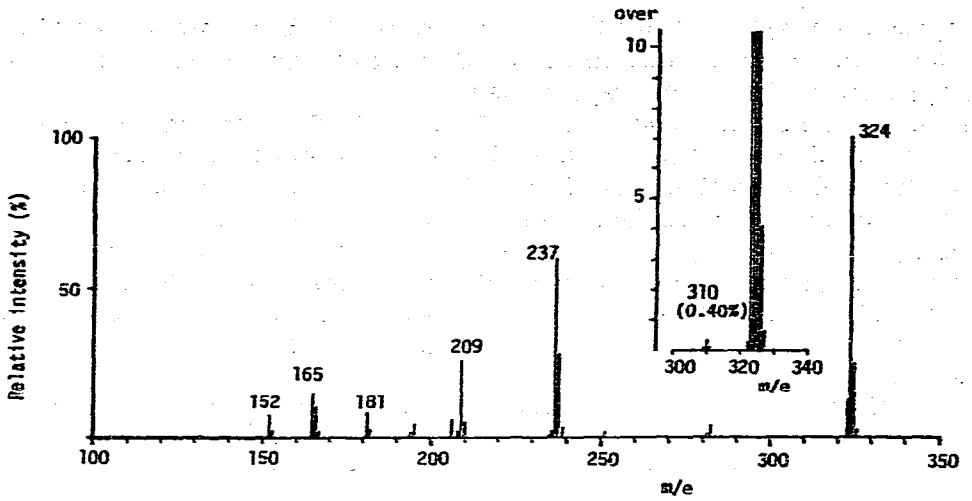


Fig. 3. Mass spectrum of DD-3505 *n*-propyl ester (IV).

and partly regenerated free oxepinac, even in mild neutral conditions, which may lead to errors in the analysis of free oxepinac. In acidic solution, decomposition of the conjugates can be disregarded for a few hours. All samples should therefore be kept deep-frozen until required for analysis.

Extraction from biological material

Isopropyl ether was found to be suitable for the extraction of oxepinac from plasma, urine or saliva. The partition coefficient of oxepinac ($C_{\text{isopropyl ether}}/C_{\text{aqueous}}$) is greater than 30 at pH 1.9 (0.1 *N* hydrochloric acid). The rates of extraction of oxepinac (I) and DD-3505 (III) with isopropyl ether from aqueous solution under acidic conditions were 98.26% and 97.26%, respectively. As solutions of oxepinac are light-sensitive, all operations must be carried out taking sufficient care to shield the solutions from light.

Derivative formation

Oxepinac (I) is a carboxylic acid and is easily converted into its *n*-propyl ester by treatment with *n*-propanol saturated with hydrogen chloride gas. The reaction kinetics are shown in Fig. 4. The reaction conditions of heating at 60° for 1 h were adequate and were chosen for the derivatization of oxepinac (I) and DD-3505 (III).

Despite the hazards associated with its use, *n*-propanol saturated with hydrogen chloride was chosen for the esterification of oxepinac (I). The mass spectrum of the methyl and ethyl esters of oxepiac gave a pattern analogous to that of the *n*-propyl ester, but when *m/e* 282 and *m/e* 296, which were the molecular ions of the methyl and ethyl esters, were monitored, these esters were found to be unsuitable for this analysis since the *m/e* 282 and *m/e* 296 ions were superimposed with the ions from the column coating. Such interference was not encountered with *m/e* 310 and *m/e* 324 ions, which were the molecular ions of the *n*-propyl esters of oxepinac and DD-3505, respectively.

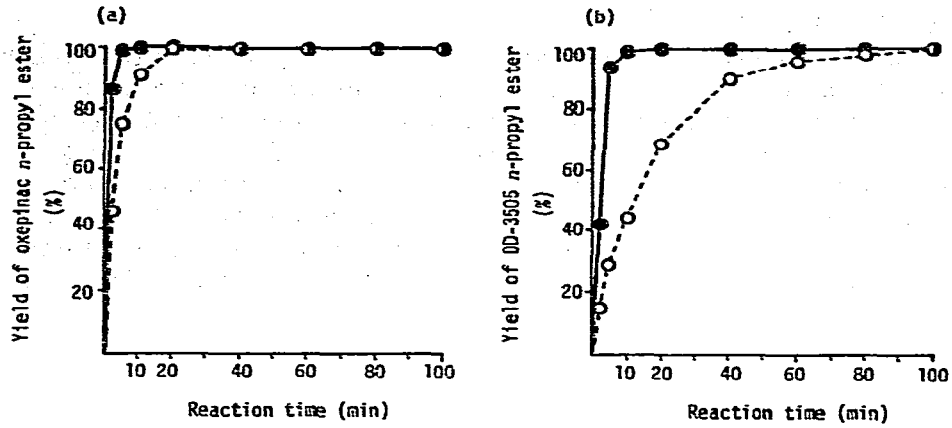


Fig. 4. Effect of temperature on the formation of (a) oxepinac *n*-propyl ester and (b) DD-3505 *n*-propyl ester. Reaction temperatures: •, 60°; ○, room temperature.

Accuracy and specificity

Typical mass fragmentograms obtained from human plasma containing oxepinac and blank plasma are given in Fig. 5. In the mass fragmentograms of extracts from blank plasma, blank urine and blank saliva, the obscured peak did not appear. Oxepinac was detected specifically. The concentration of oxepinac was determined from the peak height ratio of oxepinac *n*-propyl ester (II) to internal standard *n*-propyl ester (IV), and from response factor obtained by analysing, in parallel with the unknown samples, blank plasma, blank urine, or blank saliva to which had been added oxepinac as well as internal standard. The peak height ratio was linear over the range 1—1000 ng/ml in plasma, urine or saliva with a correlation factor of 0.997%. The overall recovery of oxepinac in this procedure was about 93%. For the lowest concentration, the precision is ±5% (n = 5) and the results are accurate to within 7%. This analytical method was so sensitive, that we could also detect the concentration of oxepinac in saliva and plasma-water in man after administrating of oxepinac.

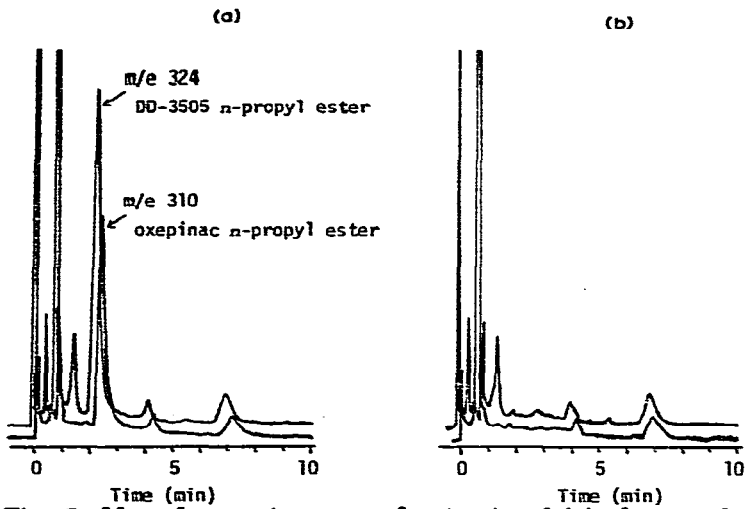


Fig. 5. Mass fragmentograms of extracts of (a) plasma of a human subject treated with oxepinac and (b) blank human plasma.

Application of the method

The utility of these methods was demonstrated by applying them to clinical experiments with human volunteers receiving single or multiple doses of oxepinac. In a typical experiment, healthy volunteers were given a tablet containing 25 mg of oxepinac. The average peak plasma level of five volunteers taking a 25-mg dose was 2.31 μg/ml. The average urinary excretion of oxepinac and acyl glucuronide during the 24 h after administration was 6.01 mg and 14.00 mg, respectively. The average peak saliva level of five volunteers taking a 100-mg dose was 25.8 ng/ml. Representative results are shown in Figs. 6—8.

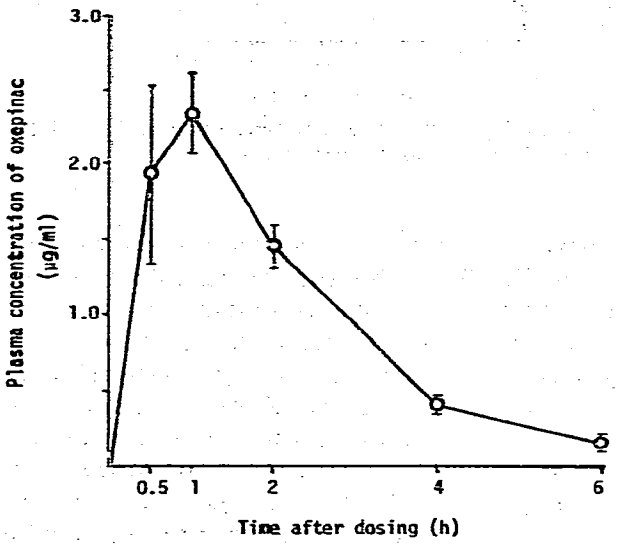


Fig. 6. Plasma levels of oxepinac in human subjects after an oral dose of 25 mg ($n = 5$).

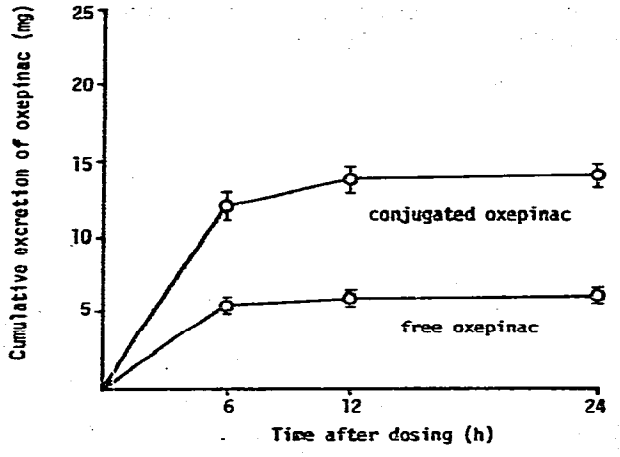


Fig. 7. Cumulative urinary excretion of free and conjugated oxepinac in human subjects after an oral dose of 25 mg ($n = 5$).

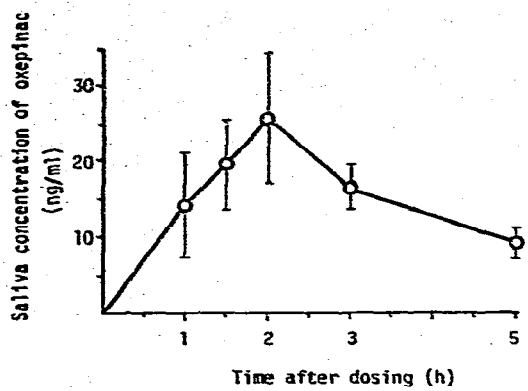


Fig. 8. Saliva levels of oxepinac in human subjects after an oral dose of 100 mg ($n = 5$).

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