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Note

A simple and sensitive gas–liquid chromatographic method for the determination of o,p'-DDD in biological fluids

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The compound *o,p'*-DDD [1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethane; mitotane] produces atrophy of the adrenal cortex, inhibition of adrenocorticotropic hormone, stimulation of steroid production, and alteration of liver cortisol metabolism[1,2]. *o,p'*-DDD is used in the treatment of Cushing's syndrome, secondary to adrenal carcinoma[3], or hyperfunction[4].

Although several methods have been reported which may be utilized to measure *o,p'*-DDD, most of them are used for qualitative determination of chlorinated pesticides[5–9]. The only specific method described is a spectrophotometric analysis which fails to distinguish between *o,p'*-DDD and its major metabolite, 2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)acetic acid[10]. We wish to describe a sensitive and specific gas–liquid chromatographic (GLC) method for *o,p'*-DDD determination in urine and whole blood.

MATERIALS AND METHODS

Materials

1,1-Dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane, (*o,p'*-DDD) and 1,1-dichloro-2-(*m*-chlorophenyl)-2-(*p*-chlorophenyl)ethane (*m,p'*-DDD) were obtained from Aldrich (Atlanta, Ga., U.S.A.). All reagents and solvents were of analytical reagent grade.

Gas–liquid chromatography

A Varian-Aerograph Model 2700 gas–liquid chromatograph equipped with dual hydrogen flame ionization detectors (FID) was used. The column was a glass coil (6.0 ft. × 1/8 in. O.D.) packed with 3% OV-225 on 100–200 mesh Varaport 30. The column was operated isothermally at 240°, with the injection port at 260°, and the detector at 300°. Helium was used as the carrier gas. The flow-rates of hydrogen and air were optimized so as to obtain maximum peak heights.

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Quantitative measurements

Stock solutions of *m,p'*-DDD and *o,p'*-DDD (each 1 mg/ml) were prepared and aliquots of each mixed and diluted to 50 ml with methanol to give a range of standard solutions each containing 50 μg/ml of *m,p'*-DDD as the internal standard and from 2.0 to 20.0 μg/ml of *o,p'*-DDD . A calibration graph was obtained by injecting 3- to 5-μl aliquots of the appropriate standards and plotting peak height ratios against concentrations.

Extraction procedure

To 1 ml of whole heparinized blood, 4 ml of water, 0.1 ml of 0.1 *N* HCl and 20 ml of diethyl ether are added. The solution is shaken mechanically in a stoppered test tube for 20 min. The mixture is centrifuged for 10 min at 2000 rpm. The organic phase is separated and then extracted twice with one volume of saturated $NaHCO_3$. To the diethyl ether solution is added 0.5 ml of the internal standard solution (1 mg *m,p'*-DDD/100 ml). The solution is then evaporated to dryness. The residue is carefully dissolved in 0.1 ml of methanol and a 3- to 5-μl aliquot is injected into the gas chromatograph.

Essentially the same procedure is followed for urine samples. To 5.0 to 10.0 ml of urine is added enough 5 *N* HCl to bring the pH value of the urine to 2, followed by the addition of 20 ml of diethyl ether. The remainder of the procedure is the same as that described for blood above.

RESULTS

Performance of the analytical procedure

Symmetrical chromatographic peaks were obtained for both compounds (Fig. 1) and the retention time for *o,p'*-DDD is 16 min and that for the internal stan-

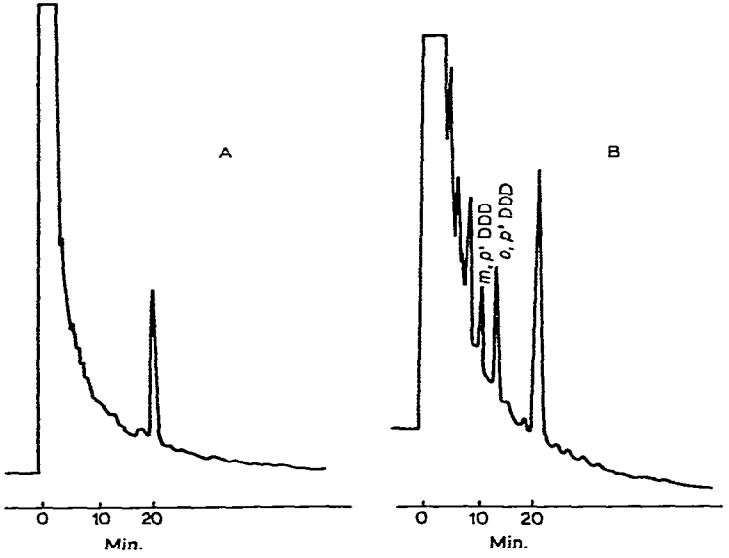


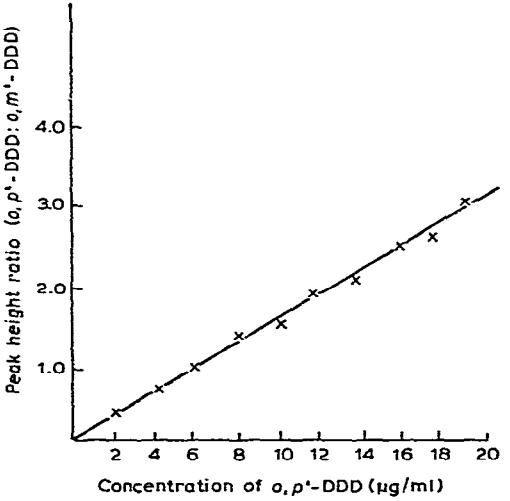
Fig. 1. Gas chromatograms of (A) blank blood extract and (B) extract of blood taken from a rabbit receiving *o,p'*-DDD.

TABLE I

RECOVERY OF *o,p'*-DDD AFTER *IN VITRO* ADDITION TO RABBIT BLOOD AND URINE

Amount added (μg)	*Found* (μg ± Standard Error)*	*Recovered (% ± Standard Error)*
To Blood		
2.0	1.63 ± 0.07	81.50 ± 3.50
6.0	4.85 ± 0.02	80.83 ± 0.33
8.0	6.70 ± 0.09	83.75 ± 1.00
10.0	8.03 ± 0.11	80.30 ± 1.10
12.0	9.52 ± 0.21	79.33 ± 1.75
14.0	11.41 ± 0.53	78.75 ± 3.78
16.0	13.67 ± 0.32	85.43 ± 2.00
18.0	14.11 ± 0.27	78.38 ± 1.50
20.0	15.91 ± 0.50	79.55 ± 2.50
		Mean 80.06 ± 1.82
To Urine		
2.0	1.91 ± 0.08	90.95 ± 4.00
4.0	3.53 ± 0.05	88 25 ± 1.25
6.0	5.84 ± 0.08	97.33 ± 1.36
8.0	7.82 ± 0.09	97.75 ± 1.16
10.0	9.58 ± 0.01	95.80 ± 0.11
12.0	11.51 ± 0.43	95.91 ± 3.58
14.0	13.01 ± 0.21	92.92 ± 1.50
16.0	14.92 ± 0.52	93.25 ± 3.25
18.0	16.15 ± 0.67	89.72 ± 3.72
20.0	19.18 ± 0.48	95.90 ± 2.40
		Mean 93.78 ± 2.23

* Each value is the mean of three determinations.



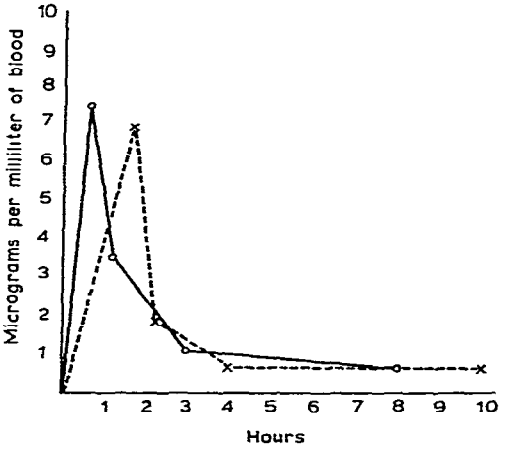


Fig. 2. Calibration curve for the determination of *o,p'*-DDD in blood and urine extracts containing 2–20 μg of drug.

Fig. 3. *o,p'*-DDD concentrations in two rabbits following a single oral dose of 300 mg.

dard, *m,p'*-DDD, is 12 min. The calibration curve for *o,p'*-DDD is constructed over the range 2.0–20.0 μg and is shown in Fig. 2. The values obtained result in a linear plot which passes through the orgin.

The recoveries from the rabbit blood and urine samples are constant in the range examined, with mean values of 80 and 93% for blood and urine, respectively (Table I). No interference from endogenous substances was noted.

A skilled technician should be able to perform these procedural steps for 15 to 20 blood or urine samples plus an internal calibration graph in 4 h. The method has been applied in our laboratory to the routine determination of *o,p'*-DDD in rabbit blood. Preliminary results indicate that *o,p'*-DDD is rapidly absorbed when given to rabbits orally, shows a peak blood level in 1–2 h (Fig. 3), and is barely detectable at approximately 4 h after administration. Apparently no *o,p'*-DDD is excreted in the urine after a single oral dose at 100 mg/kg of *o,p'*-DDD.

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