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Note

Gas chromatographic determination of (*o*-methyl-α-phenylbenzyloxy)acetic acid levels in human serum following therapeutic doses of orphenadrin (Disipal®)

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Orphenadrin [N,N-dimethyl-2-(*o*-methyl-α-phenylbenzyloxy)ethylamine] is a centrally acting skeletal muscle relaxant initially used as a therapeutic agent for the symptomatic treatment of Parkinson's disease. Medication with anti-cholinergic anti-parkinsonian drugs is also required for psychiatric patients medicated with long-acting (depôt) neuroleptics [1].

The metabolism, distribution and excretion of orphenadrin in man has been studied by several authors [2, 3]. Using [^{3}H]orphenadrin Ellison et al. [2] detected several metabolites by means of thin-layer chromatography. One of the major metabolites of orphenadrin, (*o*-methyl-α-phenylbenzyloxy)acetic acid (OMBOA), excreted in the urine as the glucuronide, accounted for about 13% of a 100-mg oral dose of [^{3}H]orphenadrin ingested by a volunteer. One or more of the metabolites of orphenadrin was shown to interfere in the determination of thyroxin in serum [4, 5] or possibly even affect thyroid function; for this reason we planned to investigate the influence of a prolonged use of orphenadrin on the thyroid function of psychiatric patients. As we planned to correlate serum levels of the most important metabolite of orphenadrin with thyroid function, the development of a method for the determination of OMBOA was necessary.

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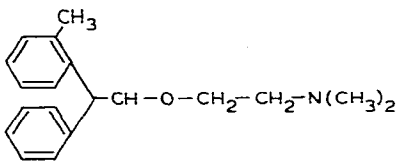
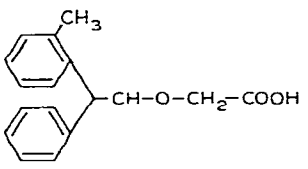
MATERIALS AND METHODS

Reagents

N,N-Dimethyl-2-(*o*-methyl-α-phenylbenzyloxy)ethylamine · HCl (orphenadrin) and (*o*-methyl-α-phenylbenzyloxy)acetic acid (OMBOA) were kindly supplied by Gist-Brocades, Haarlem, The Netherlands (Fig. 1). The internal standard, primidone, was obtained from ICI-Holland (Rotterdam, The Netherlands); a solution of 2.5 μg/ml in redistilled chloroform was prepared. All other reagents and solvents were obtained from Merck (Darmstadt, G.F.R.).



N, N-Dimethyl-2-(*o*-methyl-α-phenylbenzyloxy)ethylamine (Orphenadrin)



(*o*-methyl-α-phenylbenzyloxy)acetic acid (OMBOA)

Fig. 1. Chemical structures of orphenadrin and OMBOA.

Extraction procedure

A 1.0-ml volume of serum, acidified with 0.2 ml of 1.0 *M* HCl, was extracted for 1 min on a Vortex mixer with 10.0 ml of internal standard solution in chloroform. After centrifugation for 5 min at 2500 *g*, 9.0 ml of the chloroform layer were transferred and evaporated to dryness at 50° and under reduced pressure. The residue was dissolved in 100 μl of absolute ethanol.

Gas chromatography

A Pye series 104 gas chromatograph equipped with a flame-ionisation detector and connected with a SpectraPhysics SP-4000 chromatography data system was used. Silanised glass columns (1.8 m × 2 mm I.D.) were packed with 3% OV-17 on Chromosorb W AW DMCS HP, 80—100 mesh (Chrompack, Middelburg, The Netherlands).

The gas chromatographic conditions were: injector block at 260°; detector at 270°; column oven at 250°; nitrogen flow-rate 40 ml/min; hydrogen and air pressure 14.5 and 13.0 p.s.i., respectively; chart speed 0.5 cm/min.

A 2.0-μl aliquot of the ethanolic solution of the extraction residue (100 μl) was injected into the gas chromatograph. The retention times of OMBOA and primidone (internal standard) under the conditions described were about 325 and 195 sec, respectively (Fig. 2a).

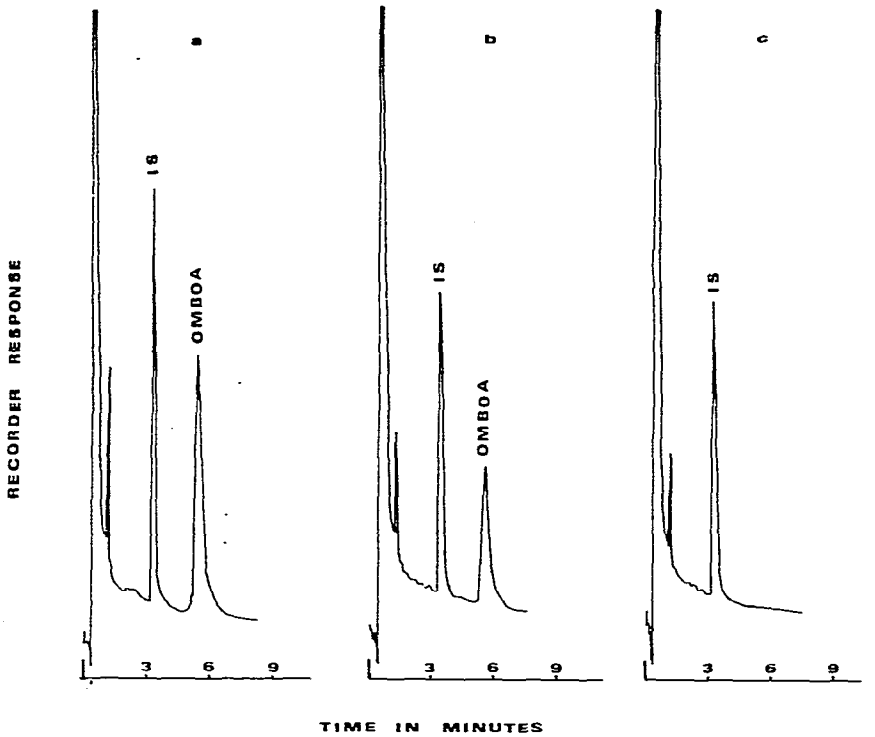


Fig. 2. Gas chromatographic analysis of OMBOA in human serum. (a) Gas chromatogram of a chloroform extract of a standard solution of OMBOA in serum (72 μg/ml). (b) Gas chromatogram of a chloroform extract of serum from a patient who ingested 300 mg orphenadrin daily for more than 1 year. (c) Gas chromatogram of human serum after addition of orphenadrin, four of its metabolites and primidone (internal standard, IS) showing the absence of interference from these compounds in the retention area of OMBOA.

RESULTS AND DISCUSSION

Accuracy and reproducibility

Calibration curves were constructed by analysing serum samples to which different amounts of OMBOA were added to yield concentrations of 12, 24, 36, 48, 60 and 72 μg/ml.

A linear relationship between the peak area response ratios of OMBOA and primidone versus concentration was observed; the slope of the linear regression line was 0.9989, thus validating the linearity of the calibration curve. This standard curve covers the concentration range of serum OMBOA in patients using therapeutic amounts of orphenadrin.

Reproducibility of the determination of OMBOA, verified by the repeated assay of the same sample of serum, yielded a mean concentration of 23.6 μg/ml with a coefficient of variation of 5.3% (n = 8).

The minimum detectable amount of OMBOA was 13 ng (signal-to-noise ratio = 3:1), thus the sensitivity limit of the assay is 0.8 μg/ml of serum. Inter-assay variability determined on consecutive days yielded a mean value of 27.3 ± 5.5% (n = 6).

Recovery

Overall recovery of OMBOA taken through the analytical procedure was determined by a comparison of the peak area ratios, obtained from a serum containing 30 μg/ml of OMBOA, with those of a standard of the same concentration injected directly. The single chloroform extraction yielded an effective recovery of 83,4% with a coefficient of variation of 2.5% (n = 6).

Specificity

Several metabolites of orphenadrin have been described by Ellison et al. [3]; besides OMBOA they identified N-methyl-2-(*o*-methyl-α-phenylbenzyloxy)ethylamine, 2-(*o*-methyl-α-phenylbenzyloxy)ethylamine, N,N-dimethyl-2-(*o*-methyl-α-phenylbenzyloxy)ethylamine-N-oxide and *o*-methylbenzhydrole.

Serum spiked with these metabolites was processed under the conditions described for the assay of OMBOA. No interference was detected, as can be seen from Fig. 2c.

Salicylic acid, the major metabolite of aspirin, was also processed under these conditions but the retention time proved to be only about 70 sec. The proposed method is therefore specific for the metabolite OMBOA.

So far, 28 serum samples of patients not receiving orphenadrin have been tested. In none of them has a peak been observed during gas chromatography which interferes with the primidone or OMBOA peaks.

Application in human subjects

Blood samples were obtained from 28 psychiatric patients treated with different amounts of orphenadrin during a period of at least 1 year. The serum was separated and stored at −25° prior to analysis.

The steady-state concentrations of OMBOA found are shown in Fig. 3 as a function of the daily dosage of the drug. The correlation between the OMBOA concentrations and the daily dose of orphenadrin proved to be poor, the coefficient of correlation being 0.6498. A representative chromatogram is shown in Fig. 2b.

From these results it can be seen that there is considerable individual variation in absorption, metabolism and/or elimination of orphenadrin in man.

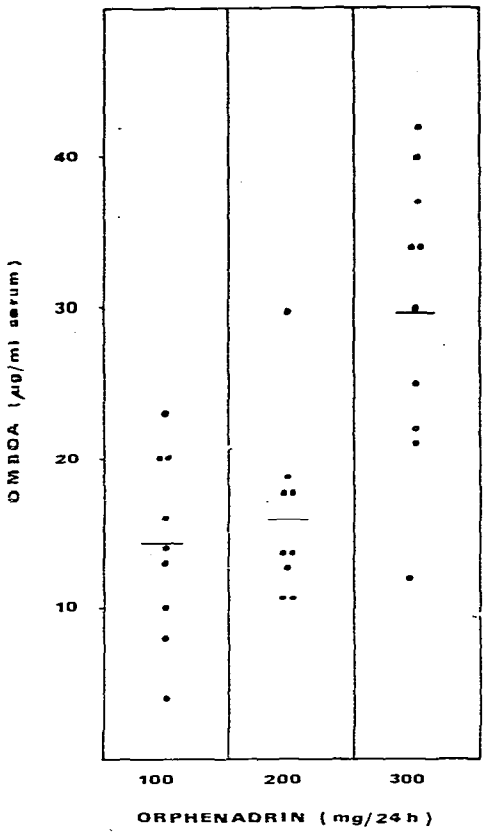


Fig. 3. Relationship between the daily dose of orphenadrin and the serum concentration of OMBOA in psychiatric patients.

CONCLUSIONS

The proposed technique permits the rapid assay of OMBOA in serum with an adequate degree of accuracy and specificity. The sensitivity of the assay (0.8 µg/ml) is sufficient to measure serum levels of OMBOA, the major metabolite, after thereapeutic doses of orphenadrin.

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