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## Note

# Quantitative gas–liquid chromatographic determination of medroxyprogesterone acetate in human plasma

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Medroxyprogesterone acetate (6-α-methyl-17α-acetoxyprogesterone; MPA) is a synthetic steroid derived from progesterone[1]; unlike natural progesterone and 17α-hydroxyprogesterone, both of which are inactive when administered orally, MPA has a strong progestational effect in both man and animals[1,2] after oral administration, and is used in the control of fertility[3].

Recent investigations in humans showed advantages in the use of high doses of MPA as a single drug in the primary treatment of hypernephroma[4] and of endometrial carcinoma[5], and in the secondary treatment of breast[6] and prostatic carcinoma[7].

A gas–liquid chromatographic (GLC) method described in a previous paper for the determination of MPA in dog plasma is not completely suitable for human plasma because of endogenous interference[8]. This paper reports a specific GLC method for the determination of MPA in human plasma. After extraction of the plasma with cyclohexane and formation of the 3-enol heptafluorobutyrate ester of the drug, MPA is determined by GLC on an OV-17 column with an electron-capture detector.

## EXPERIMENTAL

### *Standards and reagents*

MPA was kindly supplied by Farmitalia (Milan, Italy). Phenprocoumon [3-(α-ethylbenzyl)-4-hydroxycoumarin] was used as external marker after derivatization with pentafluorobenzyl bromide. Other reagents used were acetone, acetonitrile, benzene, cyclohexane (analytical reagent grade) (Carlo Erba, Milan, Italy) and heptafluorobutyric anhydride (HFBA puriss grade) (Fluka, Milan, Italy).

### *Gas–liquid chromatography*

A Carlo Erba Fractovap 2300 gas chromatograph equipped with a $^{63}Ni$ electron capture detector (ECD) was used. The chromatographic column was a glass

tube, 80 cm × 4 mm I.D., packed with 3% OV-17 on 100–120-mesh Gas-Chrom Q (Applied Science Labs., State College, Pa., U.S.A.), conditioned for 12 h at 250° (nitrogen flow-rate 40 ml/min), 4 h at 340° (without nitrogen) and 12 h at 280° (nitrogen flow-rate 40 ml/min). The operation conditions were: column temperature, 220°; injection port temperature, 250°; detector temperature, 270°; carrier gas (nitrogen) flow-rate, 40 ml/min; scavenger gas (nitrogen) flow-rate, 60 ml/min; and pulse interval for the ECD, 1 μsec.

*Mass spectrometry*

An LKB Model 9000 mass spectrometer with a gas chromatograph was used. The mass spectrometric conditions were: ionization beam energy, 70 eV; ion source temperature, 290°; accelerating voltage, 3.5 kV; and trap current, 60 μA.

The gas chromatographic conditions were as described above, the carrier gas being helium at a flow-rate of 30 ml/min.

*Standard external calibration graphs*

A solution of MPA in acetone (1 μg/μl) was prepared and different portions covering the range 25–150 ng (three samples for each point) were dried under nitrogen. The residue from each sample was redissolved in 0.5 ml of acetone–acetonitrile (1:1) and 50 μl of HFBA were added. The tubes were shaken and the reaction allowed to proceed for 60 min at room temperature. The solutions were then evaporated to dryness under a gentle stream of nitrogen.

Each dried sample was redissolved in 100 μl of benzene, containing as an external marker phenprocoumon which had reacted with pentafluorobenzyl bromide; 1 μl of this solution was injected into the gas chromatograph.

The calibration graph was linear in the range 0.25–1.5 ng per microlitre of solution injected, as shown in Fig. 1.

*Plasma preparation*

Plasma from patients was prepared from 10 ml of venous blood which was drawn into plastic tubes, containing 5 I.U. of heparin, and centrifuged at 3000 *g*.

*Internal calibration graphs*

Increasing amounts of MPA from 5 to 50 ng/ml of plasma were added to 4 ml of human plasma and diluted to 5 ml with distilled water. The samples were extracted twice with 10 ml of cyclohexane for 20 min by shaking them in a horizontal position. After extraction, each sample was centrifuged at 885 *g* for 15 min; a total of 16 ml of the organic phase were transferred and evaporated to dryness. Each residue, redissolved in 0.5 ml of acetone–acetonitrile (1:1), was treated with 50 μl of HFBA, as described above.

## RESULTS AND DISCUSSION

The reaction of MPA with HFBA was complete within 60 min at room temperature. The identity of the reaction product was checked by GLC coupled with mass spectrometry (GLC–MS) and Fig. 2 shows the mass spectrum obtained, corresponding to MPA heptafluorobutyrate.

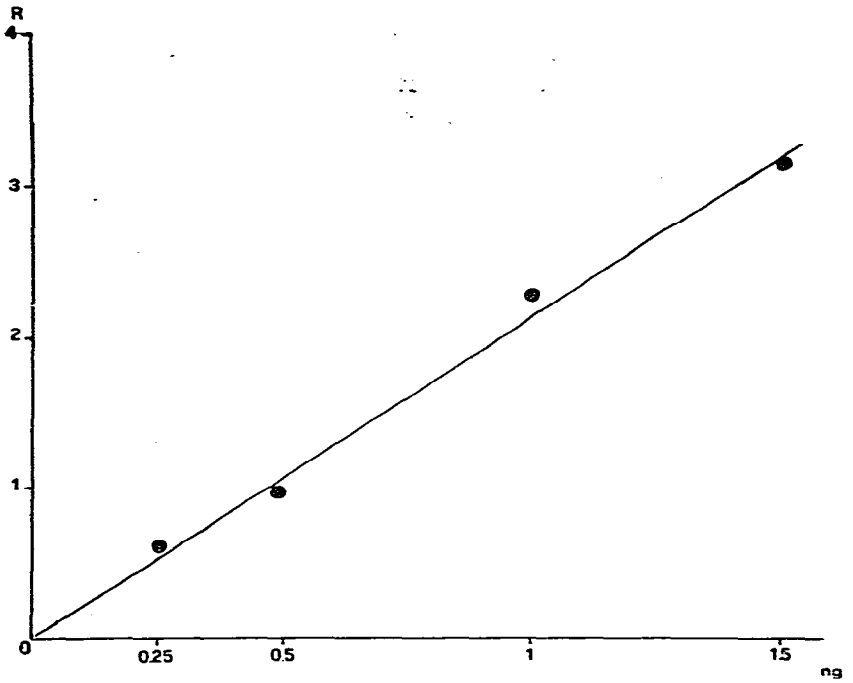


Fig. 1. External calibration graph for MPA. R is the ratio between the peak areas of MPA-heptafluorobutyrate and the external marker phenprocoumon pentafluorobenzyl bromide.

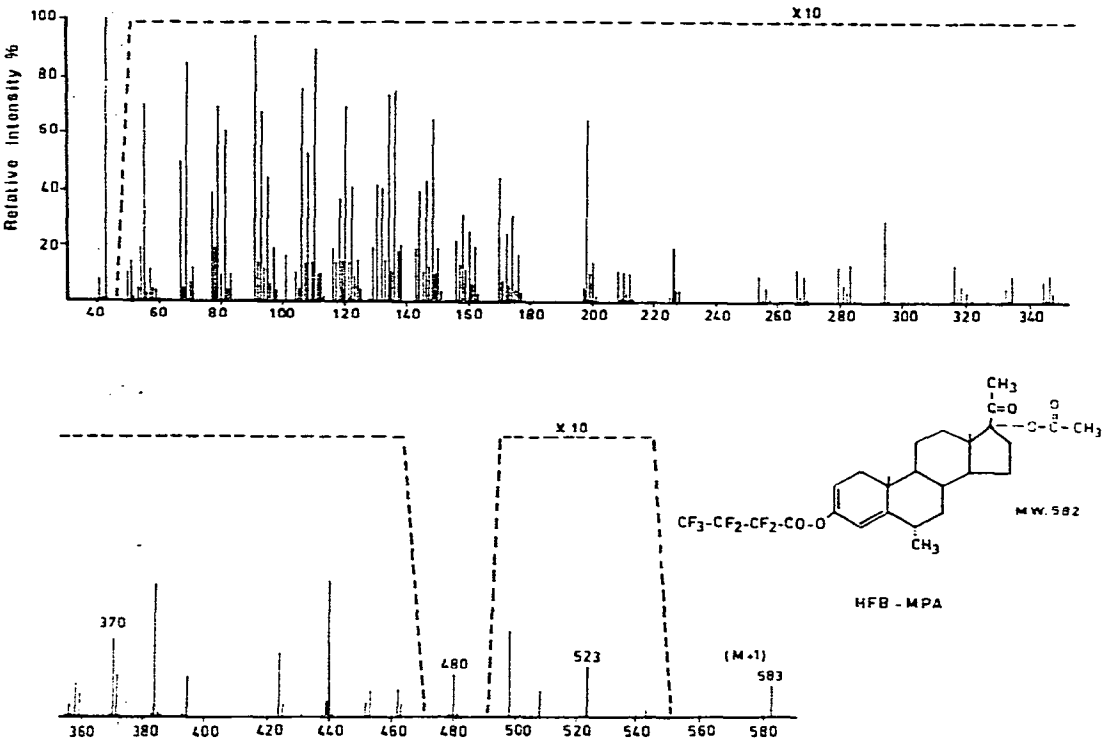


Fig. 2. Mass spectrum of MPA heptafluorobutyrate (MPA-HFB) obtained by GLC–MS.

Fig. 3 shows a typical gas chromatogram of the heptafluorobutyrate derivative of MPA and its external marker, obtained after plasma extraction and further derivatization. It shows a large peak which was also seen in the control samples and was identified by GLC–MS as the cholesterol heptafluorobutyrate derivative. Because its plasma concentration is high and the GLC retention time is very near to that of MPA-HFB, it was not possible to measure less than 5 ng/ml in plasma. Unfortunately,

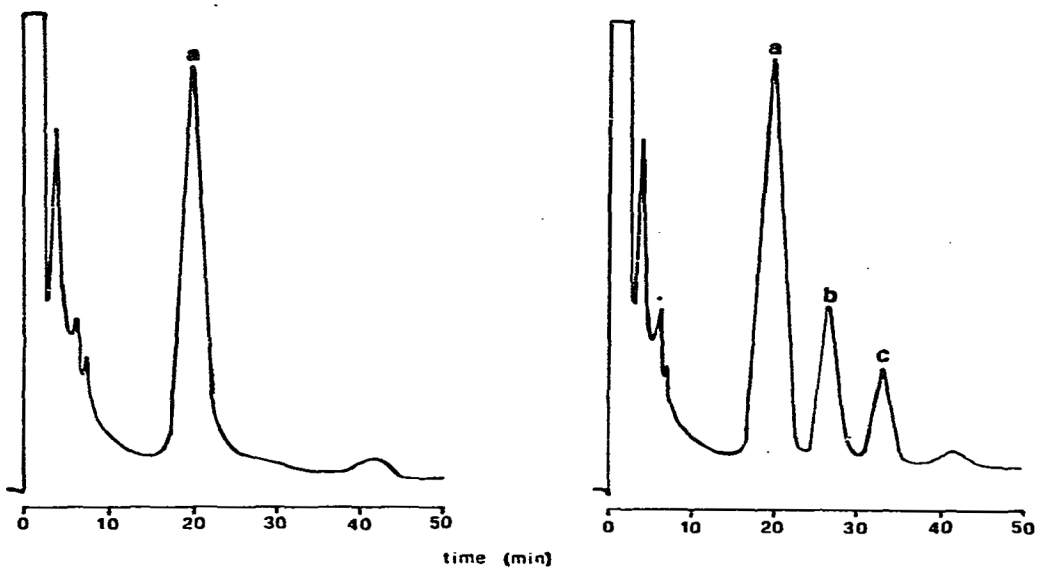


Fig. 3. Right: gas–liquid chromatogram (ECD) of human plasma extracts. Peak a corresponds to cholesterol heptafluorobutyrate, peak b to MPA heptafluorobutyrate and peak c to phenprocoumon pentafluorobenzyl bromide, used as external marker. Left: gas–liquid chromatogram of the corresponding control sample.

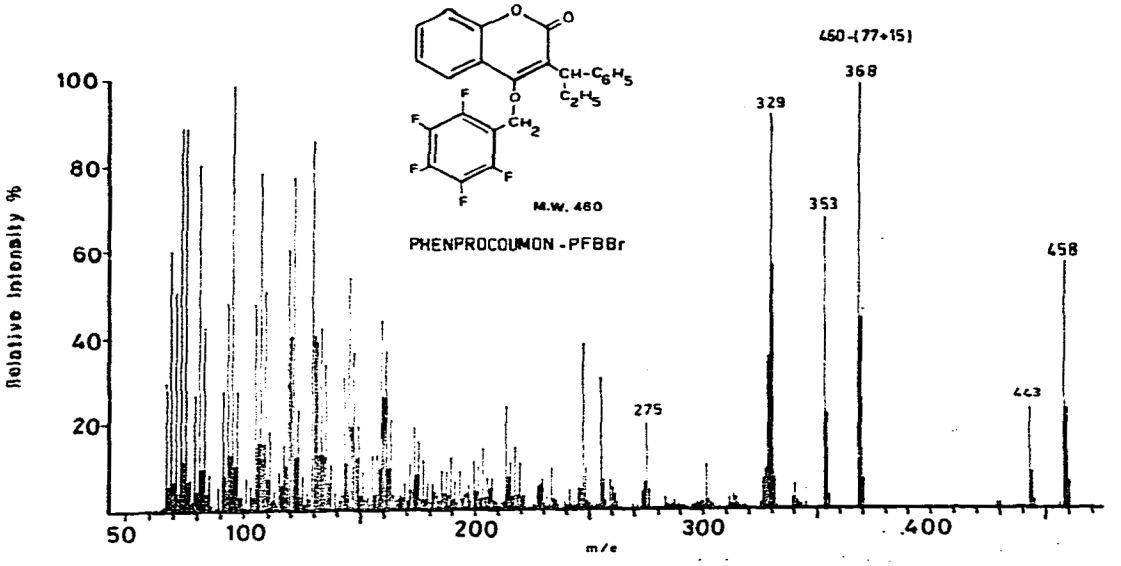


Fig. 4. Mass spectrum of phenprocoumon pentafluorobenzyl bromide obtained by gas GLC–MS.

purification was not possible, because MPA is easily extracted at any pH and has a chemical structure similar to that of cholesterol. The calibration graph obtained by plotting the ratio of the peak area of MPA-HFB to that of phenprocoumon pentafluorobenzyl bromide (mass spectrum shown in Fig. 4), its external marker, against a known concentration of MPA added to the plasma is shown in Fig. 5. Linearity

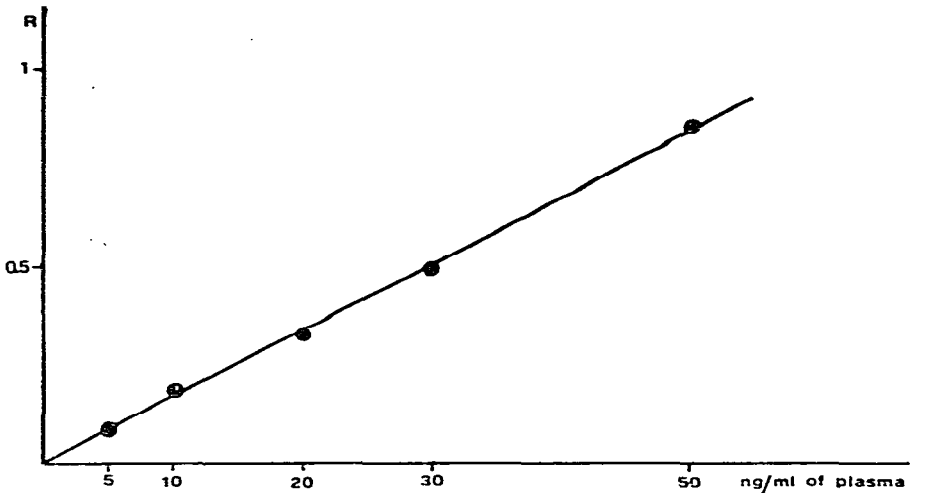


Fig. 5. Standard calibration graph for MPA added to plasma and carried through the analytical procedure; each point represents the mean of three determinations.

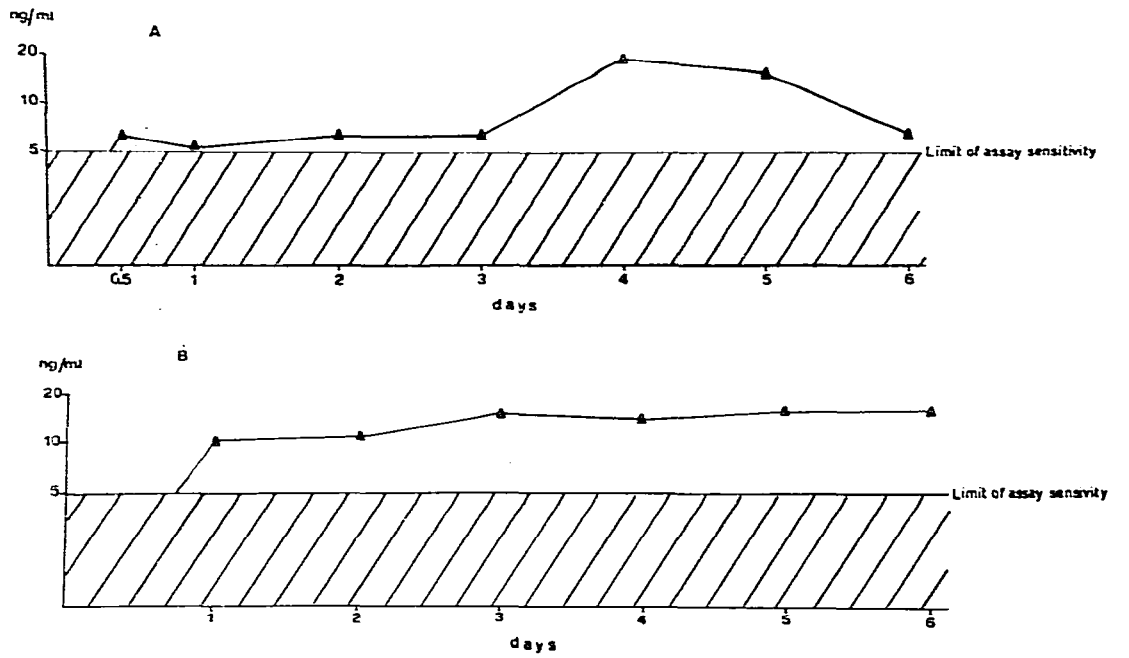


Fig. 6. Plasma levels of MPA in two patients after treatment. (A) Subject V.B., male, age 63, undergoing chronic treatment with MPA (200 mg twice daily p.o.). (B) Subject B.H., male, age 76, undergoing chronic treatment with MPA (200 mg twice daily p.o.).

was observed for concentrations between 5 and 50 ng/ml. The recovery from plasma was constant at 99 ± 4%.

The validity of this analytical procedure was tested on two subjects receiving treatment with MPA; plasma levels are reported in Fig. 6.

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