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Note

Reversed-phase high-performance liquid chromatography with isocratic elution of 6α-methyl-17α-acetoxyprogesterone (MAP) and its impurities

ERMINIO RAVINALE and ALBERTO ROSSO
Laboratory of Instrumental Analysis Farmitalia Carlo Erba, Settimo Torinese (10036), Turin (Italy)
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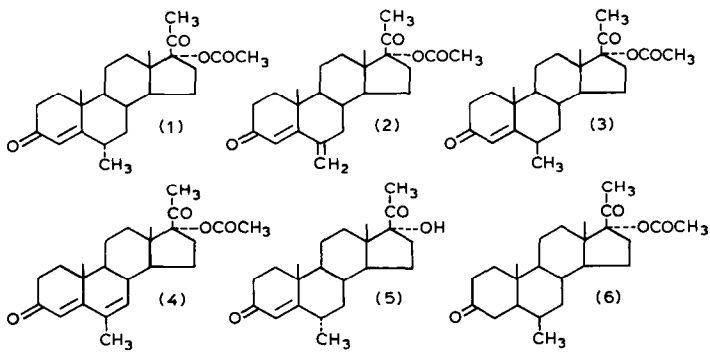
6α-Methyl-17α-acetoxyprogesterone (MAP) (1) is obtained by transfer hydrogenation of 6-methylene-17α-acetoxyprogesterone (2). Impurities in the final product can include the unreacted steroid (2), the epimer 6β-methyl-17α-acetoxyprogesterone (3) and an isomer of (2), 6-dehydro-6-methyl-17α-acetoxyprogersterone (megestrol) (4). 6α-Methyl-17α-hydroxyprogesterone (5) and 4,5-dihydro-6-methyl-17α-acetoxyprogesterone (6) obtained by hydrolysis and hydrogenation of MAP, respectively, might also be present[1]. The structures of these compounds are shown below.



The chromatographic separation of steroids 2–4 is difficult because of their similar structures and polarities. In contrast, steroids 5 and 6 are easily separated, being more and less polar, respectively, than the other steroids. Steroid 6 is difficult to detect by spectrophotometry owing to its low UV absorption.

Normal-phase[2] and a few reversed-phase[3,4] high-performance liquid chromatographic (HPLC) methods for the determination of MAP have been reported. However, none of them gave a complete separation of the above steroids. Consequently, we lack a satisfactory method for the analysis of mixtures of MAP and its reduction by-products. This paper describes a reversed-phase HPLC procedure for the isocratic separation and quantitation of MAP and all other relevant steroids.

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EXPERIMENTAL

Apparatus

The HPLC system consisted of a Hewlett-Packard Model 1090 A chromatograph equipped with .three metering pumps, an autosampler, a Model HP 1040 A variable-wavelength diode array detector, an HP 85 A personal computer controller, an HP 9121 D flexible disc drive, an HP 7470 A plotter and an HP 3392 A integrator.

Column

Chromatography was performed on a Waters Assoc. 150 × 3.9 mm I.D. stainless-steel reversed-phase Nova-Pak column, packed with octadecylsilane (ODS) (5 μm).

Chromatographic conditions

For the isocratic separation of steroids 1–5 with the ternary mobile phase tetrahydrofuran (THF)–acetonitrile (ACN)–water (12:23:65, v/v) the flow-rate was 0.6 ml/min, the column temperature 60°C, the detector wavelength 254 nm and the chart speed 0.3 cm/min (Fig. 1). For the isocratic separation of steroid 6 with the binary mobile phase acetonitrile–water (1:1, v/v), the flow-rate was 1.5 ml/min, the column temperature 60°C, the detector wavelength 200 nm and the chart speed 0.3 cm/min.

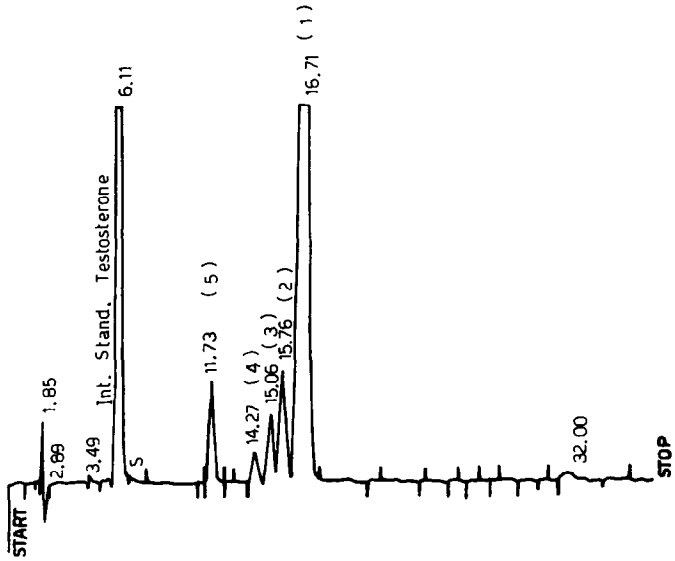

Fig. 1. Liquid chromatogram obtained from MAP + internal standard (testosterone) using a Nova-Pak reversed-phase column of 5-μm ODS (150 × 3.9 mm I.D.) with THF–acetonitrile–water (12:23:65, v/v) as the mobile phase at a flow-rate of 0.6 ml/min, with detection at 254 nm.

Quantitation

The determination of MAP was carried out with addition of testosterone as an internal standard to the sample solution (0.04%, v/v) in a 5-μl injection.

Sensitivity

The sensitivity for detection of impurities with the ternary mixture was 0.3% for the peaks of 2, 3 and 5 at 254 nm, 0.8% for the megestrol peak (4) at 254 nm and 0.3% at 292 nm (maximum absorption), and with the binary mixture it was 0.5% for the peak of 6 at 200 nm.

Precision

For the determination of MAP by the internal standard method the precision is 0.95% ($p = 0.05$).

RESULTS AND DISCUSSION

Using acetonitrile–water (1:1, v/v), the MAP peak was separated from impurities 2–4, which were fused into a single peak, while impurity 5 was resolved (Fig. 2).

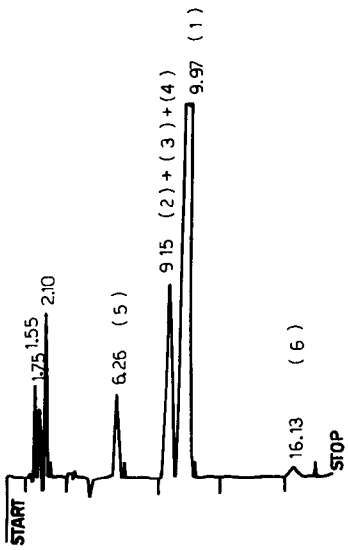

Fig. 2. Liquid chromatogram obtained from MAP using the column as in Fig. 1 with acetonitrile–water (1:1, v/v) as the mobile phase at a flow-rate of 1.5 ml/min, with detection at 254 nm. The peak of 6 was detected at 292 nm with poor sensitivity.

As the detection of 6 was not possible at 254 nm and was very poor at 280 nm (typical maximum absorption band for saturated ketones)[5], we undertook wavelength screening to find the optimal conditions for detection. The far-UV region (200 nm) gave the best result (Fig. 3).

THF–water (35:65, v/v) separated the MAP epimer (3) from the two single peaks formed by 4 + 5 and 1 + 2 (Fig. 4).

To improve the selectivity of the separation we tried the ternary mixture THF–acetonitrile–water[6]. The proportions 12:23:65 (v/v) allowed the complete resolution of all six steroids. However, owing to the cut off of the THF, 6 could not be

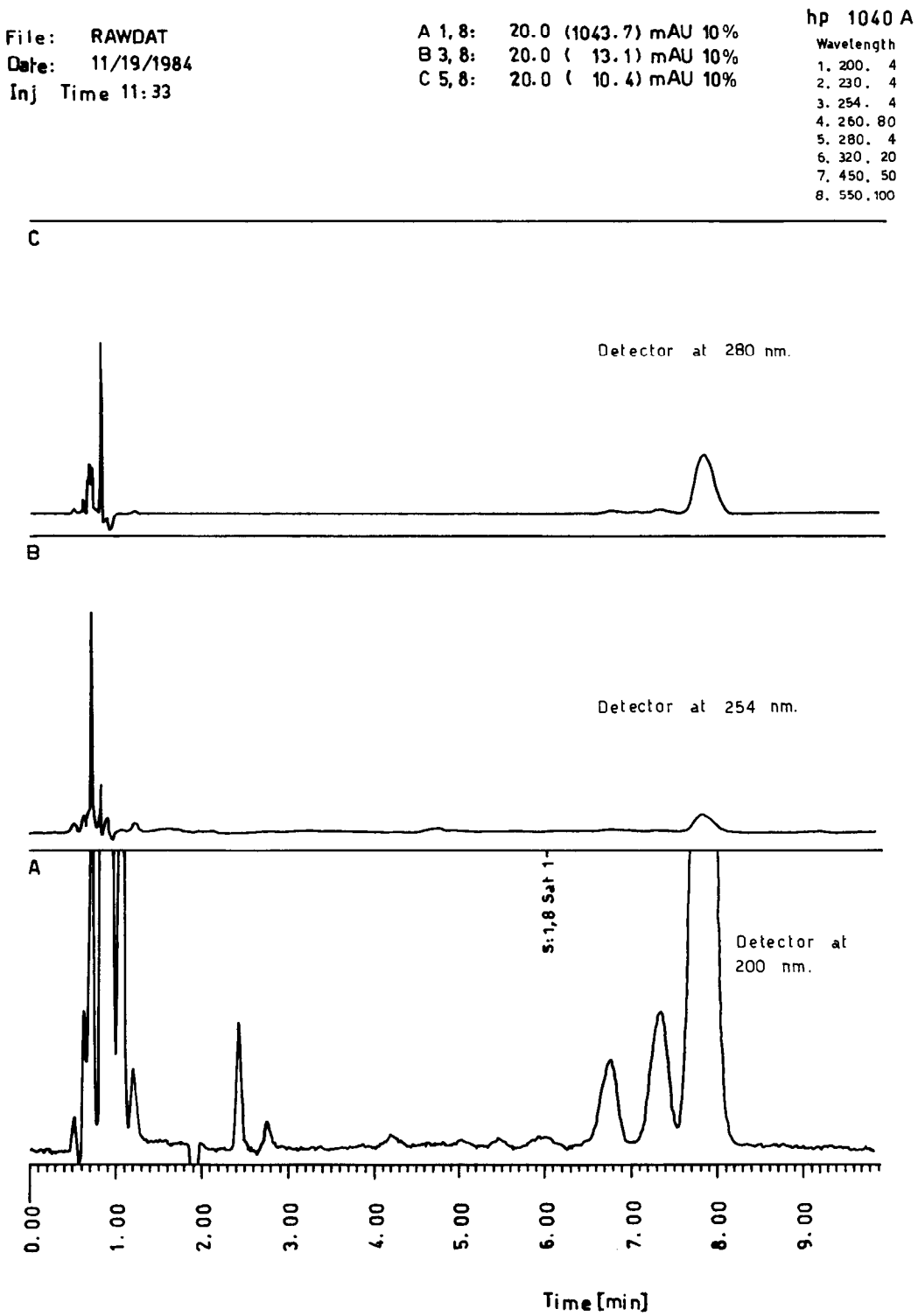

Fig. 3. Liquid chromatogram obtained from steroid 6. Conditions as in Fig. 2, with multi-signal detection at 254, 292 and 200 nm.

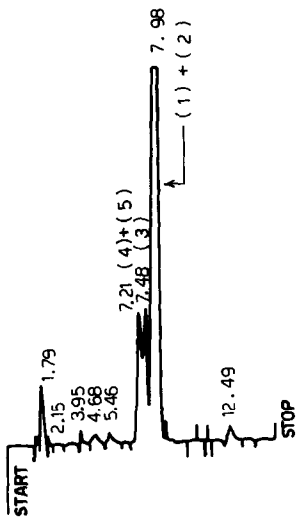

Fig. 4. Liquid chromatogram obtained from MAP using the column as in Fig. 1 with THF–water (35:65, v/v) as the mobile phase at a flow-rate of 0.5 ml/min, with detection at 254 nm.

detected at 200 nm; therefore, it should be detected using acetonitrile–water as the mobile phase.

In every run, the identity and purity of each peak were checked by spectroscopic tests carried out with an HP 1040 A diode array detector. Only with THF–acetonitrile–water as the mobile phase were single peaks obtained for all the steroids, confirming a satisfactory resolution. The mass reaction and solid samples of MAP, both containing the above-mentioned impurities, could be successfully analysed using the HPLC method described here. Hence it is particularly suitable for application in process and quality control.

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