Analysis of Testosterone Metabolites using HPLC

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Abstract

Cytochrome P450 (CYP) is a family of haemoproteins and oxygenase. CYP plays an important role in the detoxification of drugs, mutagen, and carcinogen. They exist in multiple forms (or isoenzymes) which show broad substrate specificity and differing catalytic activity. The CYP isoenzyme shows the stereospecific hydroxylation of steroid nucleus, therefore, the metabolism of testosterone was used as a probe for CYP isoenzyme activities.

In this Application Brief we demonstrate the highly precise analysis of testosterone and its metabolites.

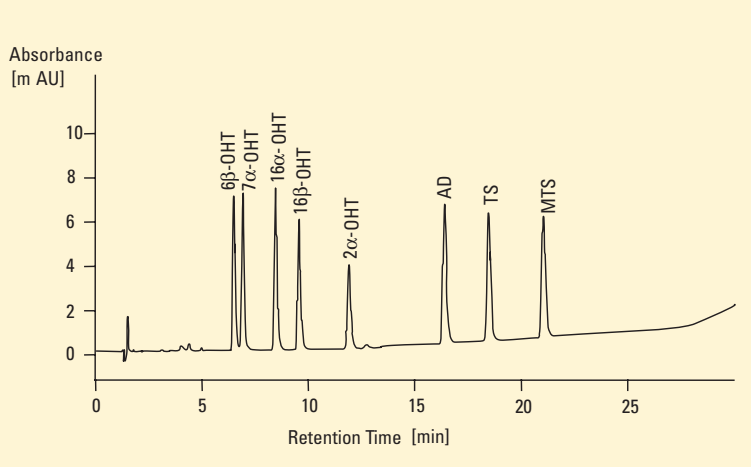

Figure 1
Chromatogram of testosterone and its metabolites
(standard solution - each peak contained 20 ng steroid)

Conditions

Column:
150 x 4.6 mm, 3 µm
Cacellpak C_{18} UG120 (Shiseido)
Mobile phase:
A = water
B = CH_3OH, C, CH_3CN
Column compartment:
40 °C
Injection vol.:
25 µl
Detector:
diode-array detector
wavelength 245 nm
reference 450 nm

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Analyzed compounds

The following compounds were analyzed in this applicaton: testosterone (TS), androstenedione (AD), methyltestosterone (MTS), 2α- 6β- 7α-, 16β- hydroxytestosterone (2α-, 6β-, 7α-, 16α,-16β- OHT)

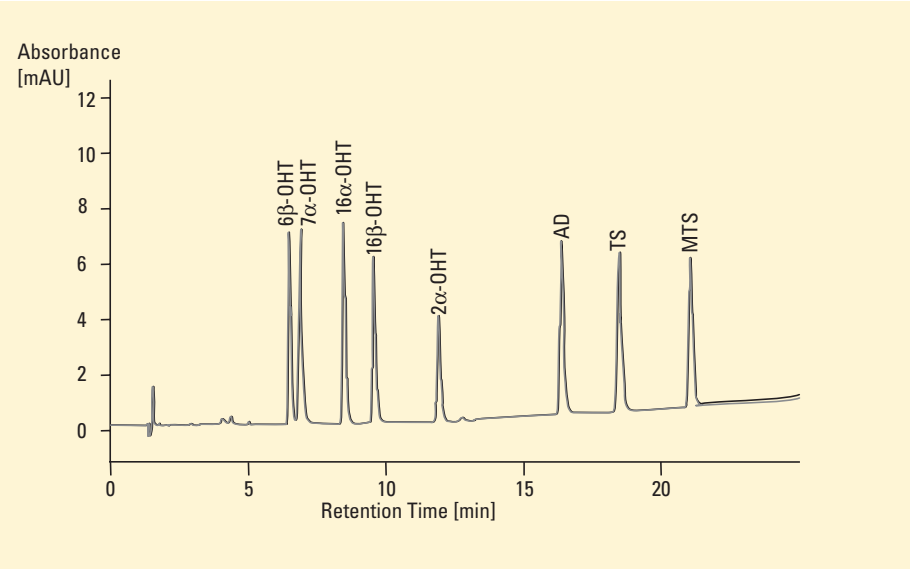

Figure 2
Overlay of 5 consecutive runs

Equipment

Agilent 1100 Series
- vacuum degasser
- quaternary pump
- autosampler
- thermostatted column compartment
- diode array detector, Agilent ChemStation + software

HPLC method performance

Limit of detection:
0.18 - 1.33 ng with S/N = 3
RSD of peak area:
0.11 - 0.78 %
RSD of retention time:
0.04-0.11 %

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Released 04/98
Publication Number 5967-5596E

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