

## Simultaneous Analysis of Testosterone and Metabolites

After being ingested, drugs normally undergo metabolism by cytochrome P450 (CYP) in the liver. CYP is an enzyme that converts foreign substances introduced *in vivo* into forms that are easily excreted *in vitro*. Many molecular species of CYP have been identified, but among these, 9 species, CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4, are primarily involved in drug metabolism. In particular, **CYP3A4** metabolizes the majority of commercial medicines.

In this study, analysis examples of testosterone, an indicator substrate of **CYP3A4**, and **6 $\beta$ -hydroxytestosterone**, a metabolite produced from testosterone, will be introduced.

\* This reaction is a metabolic reaction indicator for the activity evaluation of HLM (human liver microsomes) in the evaluation of drug metabolism.

### ◆ Simultaneous Analysis of Testosterone and Metabolites ◆

#### Sample: Testosterone (reaction solution of human liver microsomes)

Metabolites (6 $\beta$ -hydroxytestosterone and 6 $\beta$ -OH TES) produced from the reaction of testosterone, a type of steroid hormone, and commercial human liver microsomes (HLM).

#### [Pretreatment of Sample]

0.2 mg/mL	Human liver microsomes
10 mM	Kpi (pH 7.4)
200 $\mu$ M	Testosterone
<u>NADPH generating system</u>	
Total	200 $\mu$ L

Incubate at 37°C for 30 min

Stop reaction with 2 mL of CH<sub>2</sub>Cl<sub>2</sub>

Add 1 mL of 3 M NaCl

Add 20  $\mu$ L of 20  $\mu$ M Corticosterone as an internal standard

Vortex vigorously

Centrifuge at 2,000 rpm for 5 min

Collect organic layer

Dry under N<sub>2</sub> stream

Reconstitute with 200  $\mu$ L of mobile phase

Inject 10  $\mu$ L to HPLC

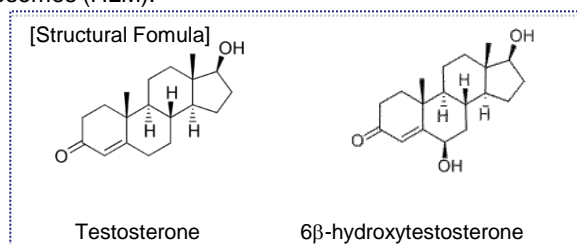
#### [LC Conditions]

Column	HITACHI LaChrom C18 (5 $\mu$ m) 4.6 mm I.D. $\times$ 150 mm HITACHI LaChrom C18 (3 $\mu$ m) 4.6 mm I.D. $\times$ 100 mm
Eluent	50% CH <sub>3</sub> OH /10 mM potassium phosphate buffer (pH 7.4)
Flow rate	1.0 mL/min
Column temperature	35°C
Detection	UV 240 nm
Injection volume	10 $\mu$ L

#### [System configuration]

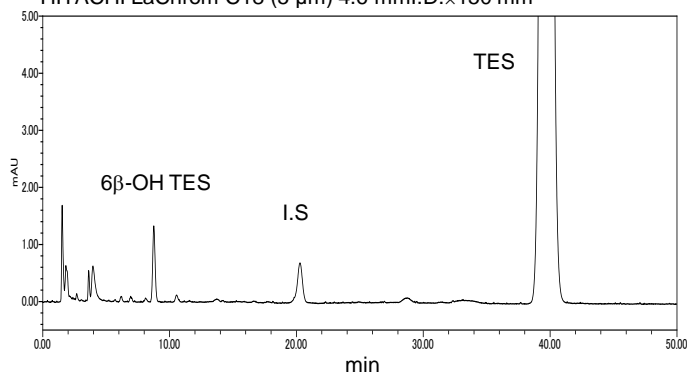
5110 Pump  
5210 AutoSampler  
5310 Column Oven  
5420 UV-VIS Detector  
Empower2 Data Processing System

Note: The data in this material are only to show examples and do not guarantee performance.

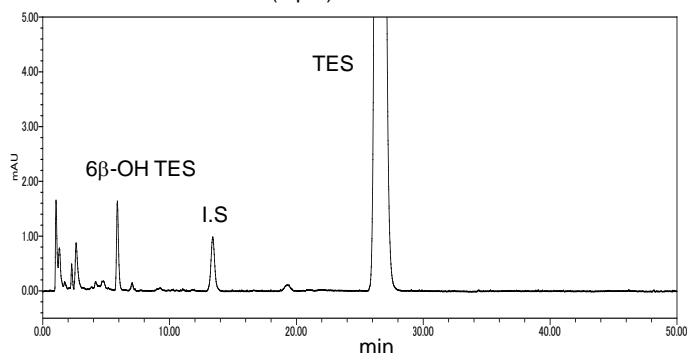


#### [Analysis Result]

\*Results using two columns which differ in size  
HITACHI LaChrom C18 (5  $\mu$ m) 4.6 mm I.D.  $\times$  150 mm



HITACHI LaChrom C18 (3  $\mu$ m) 4.6 mm I.D.  $\times$  100 mm



\*This analysis sample was provided by Miki Nakajima, Associate Professor of College of Medical, Pharmaceutical, and Health Science School of Pharmacy at Kanazawa University