

Chromaster

- Simultaneous analysis of chlorzoxazone and its metabolites -

Ingested drugs are typically metabolized by cytochrome P450 (CYP). CYP is a superfamily of enzymes that convert foreign substances taken up in the body into a form(s) that can easily be excreted. There is a large number of CYP isoforms. Among these, nine isoforms (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) are involved in the metabolism of drugs in human. This sheet describes an analysis of chlorzoxazone 6-hydroxylation, which is a marker activity of CYP2E1.

* This reaction is one of the probe activities in human liver microsomes (HLM).

Detection of chlorzoxazone and its metabolites

Sample: Incubation mixture including HLM and chlorzoxazone

* Metabolite: 6-Hydroxychlorzoxazone

* Chlorzoxazone: Central muscle relaxant

[Sample preparation method]

0.2 mg/mL	Human liver microsomes
50 mM	KPi (pH 7.4)
200 μ M	Chlorzoxazone
<u>NADPH generating system</u>	
Total	200 μ L

Incubate at 37°C for 30 min

Stop reaction with 100 μ L of acetonitrile

Add 20 μ L of 0.1 mM coumarin as an internal standard

Centrifuge at 15,000 rpm for 5 min

Inject 10 μ L of supernatant to HPLC

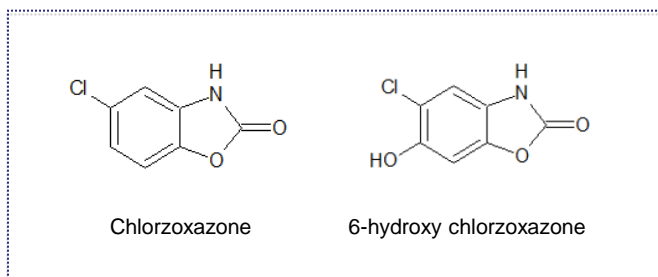
[System configuration]

Chromaster 5110 Pump
Chromaster 5210 AutoSampler
Chromaster 5310 Column Oven
Chromaster 5410 UV Detector
Empower2 Data Processing System

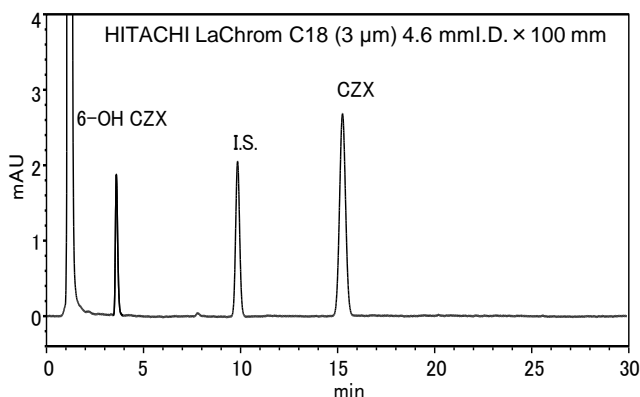
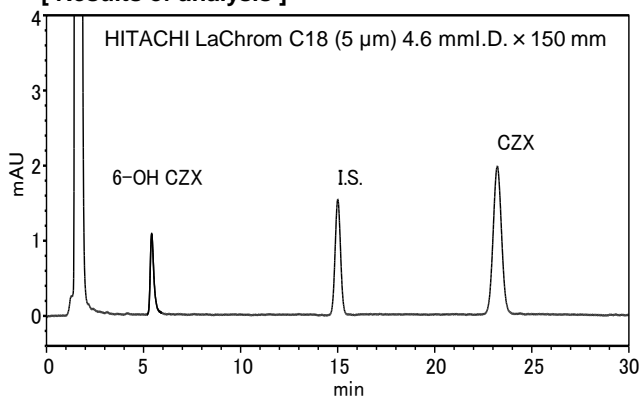
[LC conditions]

Column:	LaChrom C18 (5 μ m) 4.6 mm I.D. \times 150 mm
	LaChrom C18 (3 μ m) 4.6 mm I.D. \times 100 mm
Eluent:	(A) 50 mM KH_2PO_4 (pH 4.5),
	(B) CH_3CN , (A)/(B)=80/20
Flow rate:	1.0 mL/min
Temperature:	35°C
Detection:	295 nm
Inj. vol:	10 μ L

NOTE: These data are an example of measurement; the individual values cannot be guaranteed.



[Results of analysis]



The analytical sample courtesy of Associate Professor Miki Nakajima, Faculty of Pharmaceutical Sciences, Kanazawa University.