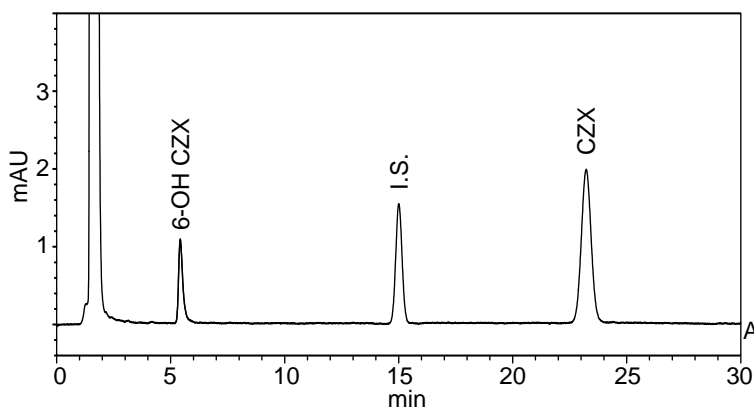


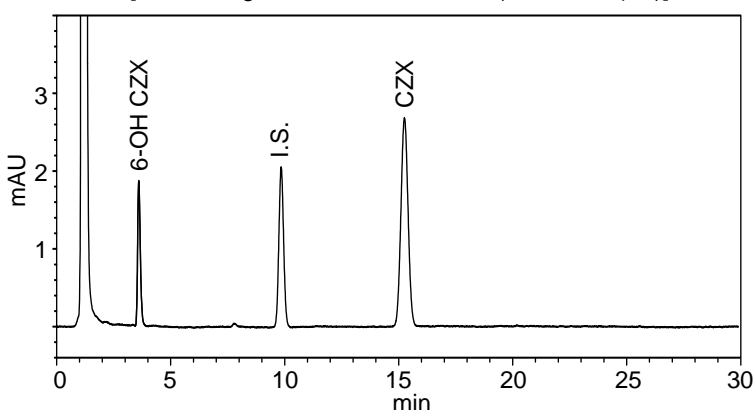
Analysis of Chlorzoxazone

In general, a drug ingested is mainly metabolized by cytochrome P450 (CYP) in liver. CYP is an enzyme which converts foreign substances taken into living bodies to the forms easily excreted. Many molecular species of CYP have been identified while 9 species among those (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) participate in drug metabolism.

An example of the analysis for chlorzoxazone, the index substrate of CYP2E1, and 6-hydroxychlorzoxazone, the metabolite produced from chlorzoxazone, is introduced here. This is the metabolic reaction which can be used as a reference for the HLM (human liver microsome) activity assessment in drug metabolism evaluation.



[Chromatogram of Chlorzoxazone (Column: 5 μ m)]



[Chromatogram of Chlorzoxazone (Column: 3 μ m)]

[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

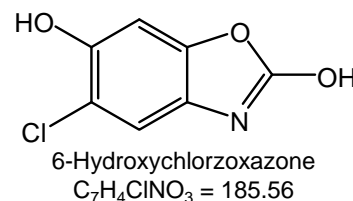
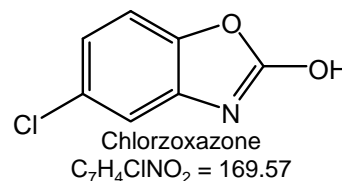
Incubate at 37 °C for 30 min

Stop reaction with 100 μ L of acetonitrile

Add 20 μ L of 0.1 mM Coumarin as internal standard

Centrifuge at 15,000 rpm for 5 min

Inject 10 μ L of supernatant to HPLC



SAMPLE 10 μ L of Std. Soln. *	PRESSURE
PACKING MATERIAL HITACHI LaChrom C18 (5, 3 μ m)	TEMPERATURE 35°C
COLUMN SIZE 4.6 mm I.D. \times 150 mm (P/N : 891-5050) (5 μ m) 4.6 mm I.D. \times 100 mm (P/N : 891-5030) (3 μ m)	SEPARATION METHOD Partition/Adsorption
ELUENT 50 mmol/L KH ₂ PO ₄ (pH 4.5) / CH ₃ CN = 80 / 20 (v/v)	DETECTOR UV 295 nm
FLOW RATE 1.0 mL/min	INSTRUMENTS Chromaster 5110 (Pump), Chromaster 5210 (Autosampler), Chromaster 5310 (Column Oven), Chromaster 5420 (UV-VIS Detector)
NOTE Sample: Human liver microsome extract (Chlorzoxazone: central muscle relaxant) This analysis sample was provided by Miki Nakajima, PhD., Division of Pharmaceutical Sciences, Graduate School of Medical Science, Kanazawa University	
KEY WORDS Bio/Medical Science/Food/Pharmaceutical, Medicine/Pharmaceutical, Biochemistry, Metabolite, Chlorzoxazone, UV-VIS Spectrometry, LaChrom C18, Chromaster, Partition/Adsorption	High Performance Liquid Chromatograph (HPLC) Sheet No. LC100021-01