Rapid LC-MS Drug Metabolite Profiling Using Microsomal Enzyme Bioreactors in a Parallel Processing Format

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SUPPORTING INFORMATION:

Experimental. Liquid Chromatography Methods: The analytical (150mm, 300 μ m I.D., 5 μ m particle size) and trapping columns (23.5mm) column were Waters capillary Atlantis dC18 and a similar procedure as reported earlier was used.¹ One 10 μ L injection for each analysis was loaded into the trapping column at flow rate of 9.25 μ L min⁻¹. Trapped metabolites were then back-flushed from the trapping column onto the analytical column at 9.25 μ L min⁻¹ using elution gradients given in the SI file, which also contains LC-MS instrumental settings.

(*Troglitazone*): 5 mins – 10% B, 3 mins – 10% to 50% B, 12 mins – 50% B, 10 mins – 50% to 90 % B, 10 mins – 90% B, 0.1 mins – 90% B to 10% B, 2min 10%B; (*Diclofenac*): 5 mins – 10% B, 3 mins – 10% to 40% B, 19 mins – 40% B, 0.1 mins – 40% to 10 % B, 3 mins – 10% B; (*Raloxifene*): 2 mins – 10% B, 13 mins – 10% to 50% B, 5 mins – 50% B, 10 mins – 50% to 10 % B. (A:10 mM acetate buffer pH 5.5, B: acetonitrile). A photodiode array detector, with a wavelength range 210-400nm, was used for sample analysis.

Electrospray ionization mass spectrometry (ESI-MS) employed a Q-TRAP 4000 Applied Biosystems (Foster City, CA) mass spectrometer operated in the positive ion mode (ESI⁺). Samples were analyzed in the single reaction monitoring (SRM) and total ion scan (TIC) modes. The ion spray voltage = 4500eV, collision energy 30 eV.

Results

Figure S1. Collision Induced Dissociation (CID) spectra for 4'-OH-DCF

Table S1. Comparing initial relative formation rates^a of DCF and TGZ metabolites in the presence of HLM-, RLM-, Bicus-CYP3A4-bioreactors or HLM in solution. Data obtained from capLC chromatograms. (Mean $\pm$ SD from 3 trials). Due to lack of metabolite standards for these drugs, the approximate reaction rates (Table S1) are reported in area•mg microsomes⁻¹•min⁻¹.

Drugs	Diclofenac		Troglitaz.	Raloxifene		
Metabolite						
Bioreact.	5OH-DCF	4'-OH-DCF	TGZQ	6-R-G	4-R-G	RAL-GSH
HLM	0.47 $\pm$ 0.06	0.17 $\pm$ 0.02	0.13 $\pm$ 0.01	0.87 $\pm$ 0.23	0.33 $\pm$ 0.10	0.17 $\pm$ 0.04
RLM	0.29 $\pm$ 0.04	0.09 $\pm$ 0.01	0.09 $\pm$ 0.02	-	-	-
Bis.-CYP3A4	0.21 $\pm$ 0.03	-	0.07 $\pm$ 0.01	-	-	-
HLM solution	0.19 $\pm$ 0.02	0.07 $\pm$ 0.01	-	0.34 $\pm$ 0.10	0.10 $\pm$ 0.04	-

^a –The ratio of metabolite vs. caffeine integrated areas of capLC chromatogram vs. time vs. amount of microsome in the bioreactors (area•mg microsomes⁻¹•min⁻¹).

Figure S2. Representative capLC chromatogram of enzyme reaction using raloxifene (R) together with silica nanospheres coated with UGT1A10 supersomes and UDP-glucuronic acid for 10min. Major product, 4'-R-glucuronide (4-R-G), shows a retention time of 9.43 min.

Reference

- (1) Tarun, M.; Bajrami, B.; Rusling, J. F. *Anal. Chem.* **2006**, 78, 624.