Introduction
The clearance of compounds eliminated by glucuronidation is typically under-estimated in vitro systems such as human liver microsomes. One explanation for this is the inhibitory effects of long-chain unsaturated fatty acids (e.g., arachidonic and linoleic acids) that are found in liver microsomal preparations. Levels of arachidonic acid in commercially available microsomes were found to be approximately 30 µM, 10% suspension, or up to 2.5-fold over 48 hours. To determine if the inhibition was due to glucuronidation, LC-MS/MS analysis with stable-labeled isotope internal standards was performed.

Materials and Methods
Materials: Human liver microsomes (Corning Cat. No. 452117), UGT Reaction Mix Solution A (Corning Cat. No. 453100), and 0.5 M Tris-HCl, pH 7.5 were obtained from Corning Life Sciences. Substrates, metabolite standards, and stable-labeled internal standards were provided by Toronto Research Chemicals. β-Estradiol, estradiol 3-β-D-glucuronide sodium salt, trifluoperazine, niflumic acid, and niflumic acid were obtained from Sigma, Santa Cruz Biotech, Toronto Research Chemicals, TLC Pharma Chem, or CDN Isotopes.

Results and Discussion
Experiments were conducted for each UGT isoform to select protein concentration and incubation times within the linear range of metabolite formation. Km, Vmax, and IC50 values were determined for each reaction using non-linear regression. The kinetic parameters obtained for each UGT isoform are listed in Table 2.

Summary and Conclusions
In vitro assays were designed to evaluate inhibition of UGT enzymes. These assays can be used to provide more confidence in the evaluation of potential in vivo DDI.

References