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A UPLC/MRM-MS method for comprehensive metabolic profiling of >50 bile acids in human and mouse samples

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Bile acids (BAs) are a group of cholesterol-derived steroid molecules, with a wide range of cytotoxicity that is associated with different pathophysiological status and multiple liver diseases. The precise and accurate quantitation of BAs in biological samples has the potential for broad applications in diagnosis, prognosis and management of liver diseases and several other health conditions. However, the presence of multiple species and isoforms poses great challenges. So far, the most comprehensive method reported quantified only 31 BAs by LC-MS/MS. In this work, we describe a robust UPLC-MRM/MS method for the simultaneous separation and profiling of >50 free and conjugated BAs in humans and mice.

Biological samples were spiked with deuterium-labelled BAs as internal standards. Acetonitrile was used to extract BAs and to remove proteins. The multiple-reaction monitoring (MRM) transitions of 51 free and conjugated BAs were optimized via direct infusion on a triple-quadrupole mass spectrometer. UPLC separation was optimized using acetonitrile-water-formic acid as the mobile phase to successfully separate 30 free, 8 glyco- and 13 tauro-BAs, including 42 isomeric species, on a 15 cm long C18 UPLC column. Scheduled MRM was applied for high-sensitivity detection and quantitation. Comparison of four sample preparation approaches indicated solid-phase extraction with phospholipid depletion showed the highest extraction efficiency. On-column analytical sensitivities of 4 to 50 femtomoles and linear ranges of 64 to 1000-fold concentrations were observed. Intra- and inter-run CVs were <8% for most BAs. The recovery tests at two standard spiking levels showed quantitation accuracies of 83.1% to 107.5% for medium-to-high abundance BAs and ≥69.5% for most low-abundance BAs. This method was used to determine the BA concentrations in fasting and non-fasting human plasma and in the plasma and liver of wild-type mice and a transgenic strain. Significant concentration changes of many BAs were observed. To detect and relatively quantitate some unknown mono-, di-, tri- and tetra-OH free and conjugated BAs, a fragmentation pattern-specific MRM approach was proposed. With this approach, for example, at least 8 unknown tetra-OH BAs were detected in mouse liver. In summary, a robust UPLC/MRM-MS method was successfully developed for comprehensive bio-analysis of free and conjugated BAs.

Biography

Jun Han is currently a research professor and a technology development leader at the University of Victoria - Genome BC Proteomics Centre, Victoria, BC, Canada. He received his Ph.D. of biological mass spectrometry in 1999 from China Pharmaceutical University and completed postdoctoral trainings in proteomics and metabolomics at University of North Carolina - Chapel Hill and Medical University of South Carolina. At the Proteomics Centre which is also part of Genome Canada-funded "The Metabolomics Innovation Centre (TMIC)", Jun Han is leading a metabolomics and tissue imaging research subgroup, with a focus on designing and developing mass spectrometry based new methods for quantitative metabolomics and MALDI tissue imaging. He has authored or co-authored more than 60 peer reviewed research papers and reviews.

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