Comparison of Bile Acid Quantitation Using UPLC/HRAM MS in Full Scan and Targeted SIM Modes

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Introduction
Bile acids (Figure 1) are produced in the liver by cytochrome P450-mediated oxidation of cholesterol. The increase of bile acid levels in human plasma can be used as a biomarker of hepatobiliary toxicity, aiding in the identification of harmful side effects of future therapeutics. Our laboratory previously developed a high throughput extraction and UPLC-high resolution accurate mass (HRAM) method to quantify up to sixteen bile acids within one injection.1-2 The assay was developed a high throughput extraction and UPLC-high resolution accurate mass (HRAM) method to quantify up to sixteen bile acids within one injection.1-2 The assay was developed previously with targeted SIM (tSIM). Here we initially compared precision and accuracy using full scan mode versus ISRM at the LLOQ of four representative bile acids. We observed better precision and resolution of peaks with the increased number of scans across the peak using full scan; therefore, we then expanded the number of analytes in the assay under full scan. The method was subsequently qualified and met all acceptance criteria.

Methodology
Sample Preparation (PPE)
Aliquot: 50.0 μL
Surrogate Matrix: Charcoal stripped human plasma
Precipitation Solvent: MeCN

LC-MS Condition
Mass Spectrometer: Thermo Fisher Q ExactiveTM Hybrid-Quadrupole-Orbitrap
Source and ionization (ESI): Column: C18, 50 x 2.1 mm, 1.7 μm
Flow Rate: 0.400 mL/min
Source Temperature: 350°C

QExactive HRAM MS Parameters
Monitoring Mode: ISRM Full Scan
Source Temp Settings: 350°C 350°C
Ionization Mode: Negative Ion Negative Ion
Source and ionization: ESI
Column: C18, 50 x 2.1 mm, 1.7 μm
Flow Rate: 0.400 mL/min
LC Program: Gradient
Source Temperature: 350°C

Results and Discussion
The chromatographic requirements were challenging. There are three groups of isobaric isomers: (1) UDCA, CDCA, HDCA and CA; (2) gDCA, gCDCA and gUDCA; (3) tDCA, HDCA and CDA; (2) gDCA, gCDCA and gUDCA; (3) tDCA, HDCA and CDA; (3) tDCA, HDCA and CDA; (2) gDCA, gCDCA and gUDCA; (3) tDCA, HDCA and CDA; (4) HDCA and CDA; (5) gDCA, gCDCA and gUDCA; (6) tCDCA and tUDCA. Because they possess the same molecular weight, they must be resolved chromatographically.

We initially compared the precision and accuracy of four representative bile acids at the LLOQ using both the full scan mode and the targeted SIM (tSIM) mode. As shown in Table 1, the %CV for ISRM went from failing (to meet acceptance criteria) to passing by moving from tSIM to full scan. The chromatographic requirements were challenging. There are three groups of isobaric isomers: (1) UDCA, CDCA, HDCA and CA; (2) gDCA, gCDCA and gUDCA; (3) tDCA, HDCA and CDA; (4) HDCA and CDA; (5) gDCA, gCDCA and gUDCA; (6) tCDCA and tUDCA. Because they possess the same molecular weight, they must be resolved chromatographically.

Concentration Name Key Functional Groups Nominal Conc (ng/mL) 1.00 3.00 200 400 1.00 3.00 200 400 1.00 3.00 200 400
%Theoretical 95.8 95.3 95.4 94.3 90.1 96.3 93 89.9
%CV 4.5 6.3 1.0 1.1 26.2 3.4 1.0 1.7

Table 1. Accuracy and Precision QC (ng/mL) with Full Scan vs Targeted SIM

Table 2. Accuracy and Precision QC (ng/mL) with Full Scan

Table 3. Accuracy and Precision QC (ng/mL) with Full Scan

Conclusion
A sensitive and selective assay for the quantitation of endogenous bile acids in charcoal stripped human plasma was developed using HRAM mass spectrometry. Full scan mode settings produced more scans across the peak compared to the ISRM settings. This allowed for improved precision and resolution from interferences peaks, especially at the LLOQ. Full scan mode was able to accurately quantify a large number of bile acids at once and can also be utilized for subsequent data mining for other compounds without data recollection.

References