# Sensitive and robust quantitation of serum bile acids using a novel quantitative kit on Stellar MS to study Crohn's disease

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## Abstract

**Purpose:** This study demonstrates a fast and sensitive quadrupole-linear ion trap mass spectrometry approach for the quantitation of 32 bile acids, including newly characterized sulfated species, using a streamlined workflow on the Thermo Scientific™ Stellar™ MS.

**Methods:** Samples (20 µL) were prepared using a precipitation and filtration workflow followed by reverse-phase liquid chromatography separation. Targeted PRM analysis was performed in negative ion mode using both HCD and CID fragmentation with matched internal standards for all analytes.

**Results:** Pooled samples analyzed across two independent sites on the Stellar MS demonstrated consistent performance, with quantitation achieved using MS2 fragment ions for 31 of 32 bile acids.

## Introduction

Absolute and accurate quantitation of bile acids in blood remains analytically challenging due to their low endogenous concentrations and structural similarity, particularly in serum matrices. These challenges are amplified in human cohort studies, such as Crohn's disease, where subtle but biologically meaningful changes in bile acid profiles are clinically relevant. Sample preparation for bile acid analysis can be labor-intensive and time-consuming, and insufficient optimization may compromise sensitivity and reproducibility. Therefore, there is a strong need for efficient, standardized sample preparation coupled with a fast, sensitive, and selective MS. Here, we address these needs using a novel bile acids kit from Move Analytical combined with the Thermo Scientific™ Stellar™ mass spectrometer, leveraging its speed, sensitivity, $MS^n$, and multiple fragmentation selectivity.

## Materials and methods

### Sample preparation

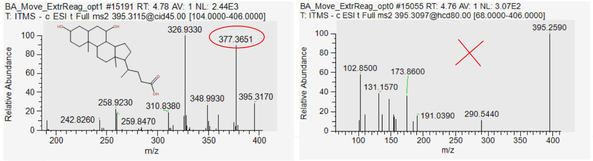
All samples were prepared and analyzed using the reagents and software provided as part of MoveKit™ BA (Move Analytical LLC). Kit performance was verified using urine, serum, and fecal sample extracts (BioIVT). Crohn's disease specimens were serum from patients and age-matched healthy controls (BioIVT). Each 96-well plate of samples was accompanied by two calibration samples in matrix, a high and low QC, and a pooled sample QC made from all samples on the plate. Labeled internal standards were sourced from Cambridge Isotope Laboratories.

Serum and urine samples were prepared following the same protocol. Fecal samples were diluted 1:10 before preparation. All samples were precipitated by dilution in high organic solution and chilling at -20 C for 10 minutes. Afterwards, samples were transferred to a glass filter plate and centrifuged. From the resulting extract, 5 µL were injected and analyzed by reverse phase LCMS using a C18 column.

### Test methods

Multi-lab study samples and method details were given to the UNC Metabolomics and Proteomics Core lab to reproduce the results collected at Thermo Fisher Scientific, San Jose. This experiment was done as a part of a larger study using the same samples and sample preparation but on Orbitrap-based instruments. Samples were prepared onsite before analysis. The two sites used slightly different C18 columns. Serum samples from Crohn's disease and healthy control patients were run using the same procedure in San Jose.

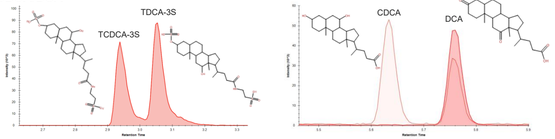
**Figure 1. Comparison of CID 45 and HCD 80 fragmentation for UDCA. Even at NCE 80 HCD does not break UDCA into significant fragments. Structure of UDCA shown.**




### Data analysis

Data was acquired using Thermo Scientific™ Xcalibur™ software with the help of the MoveApp™ software for sequence generation, real time data quality monitoring, and data processing including statistical analysis. All analytes were quantified in proportion to their exact match mass-labeled internal standard.

**Figure 2. Key chromatographic isomer separation A) TCDCA-3S/TDCA-3S B) CDCA/DCA**

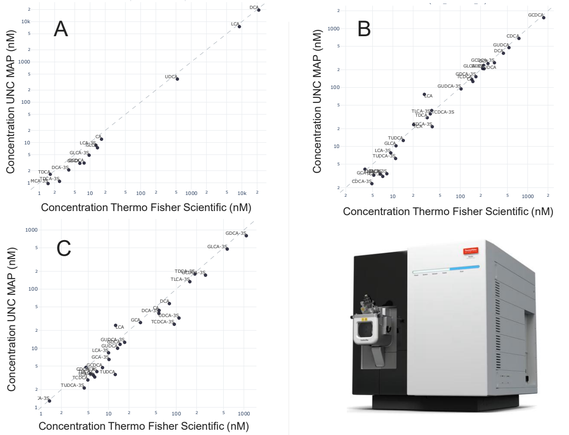



## Results

### Multi-lab study

Agreement was fairly strong between the two labs for the three samples tested, as demonstrated in Figure 3. Bile acid concentrations in feces were determined to be slightly higher by the San Jose lab, potentially in part due to using a slightly different analytical column which may not have resolved difficult chromatography or matrix peaks from the method analytes.

**Figure 3. Log-log plots comparing the average concentration of each bile acid from technical replicates (n=5) between each lab by matrix: A) feces B) serum C) urine with a y=x reference line**






### Serum from Crohn's disease patients and matched healthy controls

The volcano plot comparing Crohn's and non-Crohn's disease patients (Figure 4) indicates multiple conjugated secondary bile acids that are significant with high fold change. The literature corroborates a high depletion of TDCA in Crohn's disease patients (Sun et al.) Additionally, multiple sulfated bile acids previously undescribed in Crohn's disease serum were observed to be significant with high fold change.

**Figure 4. Volcano plot comparing Crohn's and non-Crohn's disease patients' bile acid in serum. Horizontal markings indicate a log2 fold change threshold of 1 and vertical markings indicate p<0.05. Significant bile acids are labeled. Figure adapted from MoveKit.**

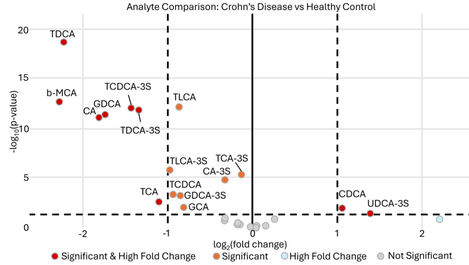
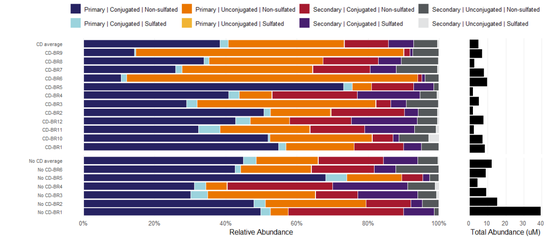



**Table 1. Analytes, their quantitative fragment, activation energy, and LLOD. LLOD was determined by measuring the concentration in a blank passed through sample preparation plus three standard deviations (n=3).**

| Analyte | Quantitative Transition (Activation Type) | LLOD (nM) |
|---|---|---|
| DCA | 391.3 > 345.3 (CID) | 7.2 |
| CDCA | 391.3 > 373.3 (CID) | 5.8 |
| UDCA | 391.3 > 373.3 (CID) | 17.5 |
| CA | 407.3 > 343.3 (CID) | 10.7 |
| b-MCA | 407.3 > 389.2 (CID) | 39.1 |
| LCA | 375.3 > 375.3 (HCD) | 11.7 |
| GUDCA | 448.3 > 74.0 (HCD) | 6.9 |
| GCDCA | 448.3 > 74.0 (HCD) | 6.0 |
| GDCA | 448.3 > 74.0 (HCD) | 2.0 |
| GCA | 464.3 > 402.3 (HCD) | 11.7 |
| GLCA | 432.3 > 74.0 (HCD) | 5.7 |
| TUDCA | 498.3 > 80.0 (HCD) | 8.4 |
| TCDCA | 498.3 > 80.0 (HCD) | 4.6 |
| TDCA | 498.3 > 80.0 (HCD) | 1.6 |
| TCA | 514.3 > 80.0 (HCD) | 1.8 |
| TLCA | 482.3 > 80.0 (HCD) | 4.6 |

| Analyte | Quantitative Transition (Activation Type) | LLOD (nM) |
|---|---|---|
| DCA-3S | 471.2 > 97.0 (HCD) | 2.7 |
| CDCA-3S | 471.2 > 97.0 (HCD) | 2.6 |
| UDCA-3S | 471.2 > 97.0 (HCD) | 4.0 |
| CA-3S | 487.2 > 97.0 (HCD) | 4.5 |
| b-MCA-3S | 487.2 > 97.0 (HCD) | 1.4 |
| LCA-3S | 455.2 > 97.0 (HCD) | 5.6 |
| GUDCA-3S | 528.3 > 448.4 (CID) | 1.4 |
| GCDCA-3S | 528.3 > 448.4 (CID) | 5.7 |
| GDCA-3S | 528.3 > 448.4 (CID) | 2.5 |
| GCA-3S | 544.3 > 464.4 (CID) | 2.3 |
| GLCA-3S | 512.3 > 432.4 (CID) | 8.8 |
| TUDCA-3S | 578.2 > 498.4 (CID) | 1.4 |
| TCDCA-3S | 578.2 > 498.4 (CID) | 3.0 |
| TDCA-3S | 578.2 > 498.4 (CID) | 3.1 |
| TCA-3S | 594.2 > 514.0 (CID) | 5.9 |
| TLCA-3S | 562.3 > 482.4 (CID) | 3.5 |

**Figure 5. Serum bile acid profiles in Crohn's Disease (CD) and non-Crohn's Disease (no CD) patients, including the average normalized profile. The left panel shows absolute abundance by bile acid category, and the right shows the sum of all bile acids in µM.**




## Conclusions

- This method demonstrates fast and accurate quantitation of 32 bile acids including 16 sulfated bile acids by one point calibration and a 9.5-minute instrument method. Performance was supported by results at a second laboratory.
- 15 out of 32 analytes were optimized to provide the best fragment intensity using CID fragmentation.
- Significant (p<0.05) differences in bile acid composition were found between the serum of Crohn's disease and healthy volunteers, including 3 sulfated bile acids.

## References

1. Remes, P.M., et al. Hybrid Quadrupole Mass Filter-Radial Ejection Linear Ion Trap and Intelligent Data Acquisition Enable Highly Multiplex Targeted Proteomics, *J. Proteome Res.* **2024**, 5476.
2. Sun R., Jiang J., Yang L., Chen L., Chen H. Alterations of Serum Bile Acid Profile in Patients with Crohn's Disease. *Gastroenterol Res Pract.* **2022** Oct 3;2022:1680008.

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