# Revolutionizing Bile Acid Detection with SCIEX

- A new targeted metabolomics method has been developed to measure bile acids (BAs) in various biological specimens such as plasma and fecal extracts, and homogenized ileal and liver tissue samples.
- This method is particularly effective at separating a subset of BA stereoisomers, which is essential for accurate quantitation. Its successful application to a variety of matrices highlights the method's versatility and reliability.
- Liquid chromatography-tandem mass spectrometry (LC-MS/MS) instruments from SCIEX were used to develop a method that has been applied here to answer research questions posed in the 3 case studies described.


## The Role of SCIEX Technology

Targeted and quantitative assays require the fast scanning and high sensitivity of a Multiple-Reaction Monitoring (MRM) workflow, and this is why the triple quadrupole (QQQ) is often favored.


ZenoTOF 7600 system

The SCIEX QTRAP systems can deliver high sensitivity and fast scanning for large quantitation panels. When analyzing complex biomolecules there may be significant benefits to acquiring high-resolution, full-scan MS/MS spectra, and the use of electron-based fragmentation strategies.

The ZenoTOF 7600 system is a powerful tool for the analysis of bile acids and other small molecules, offering high sensitivity, speed, and precision for complex sample analysis.

**1. New Q0 design**
For improved ion transmission and maintenance

**2. EAD cell**
Complimentary fragmentation with increased sensitivity using the EAD cell

**3. Zeno trap**
Improved MS/MS duty cycle gain ≥ 90%

**4. Dectector**
- 5GHz, 10 bit ADC with 40GHz TDC timing with 25psec detection rate
- High speed pulse counting to maintain resolution and mass accuracy >130Hz and over 5 orders LDR


**Main advantages:**

**Improved Duty Cycle:** The Zeno trap component controls the transfer of ions from the collision cell to the TOF accelerator. This enhances the duty cycle of the instrument, which in turn increases MS/MS sensitivity across the entire mass range.

**High Sensitivity:** With the improvements provided by the Zeno trap, the sensitivity of the instrument can be increased x 10 compared to traditional QTOF instruments, with up to 20-fold improvements observed for small molecules like lipids and metabolites, which is comparable to high-end triple quadrupole instruments.

**High-Speed Detector:** The high-speed detector can conduct over 130 MS/MS events per second whilst maintaining resolution and mass accuracy.

**Linear Dynamic Range:** The system boasts a wide linear dynamic range, spanning approximately five orders of magnitude.

**Electron Activated Dissociation (EAD):** This radical dissociation mechanism of fragmentation allows for a more detailed analysis of molecules, providing a greater level of structural characterization. Typically less efficient than Collision-Induced Dissociation (CID), this complementary fragmentation -mode takes advantage of the Zeno trap to increase the sensitivity of MS/MS fragmentation.

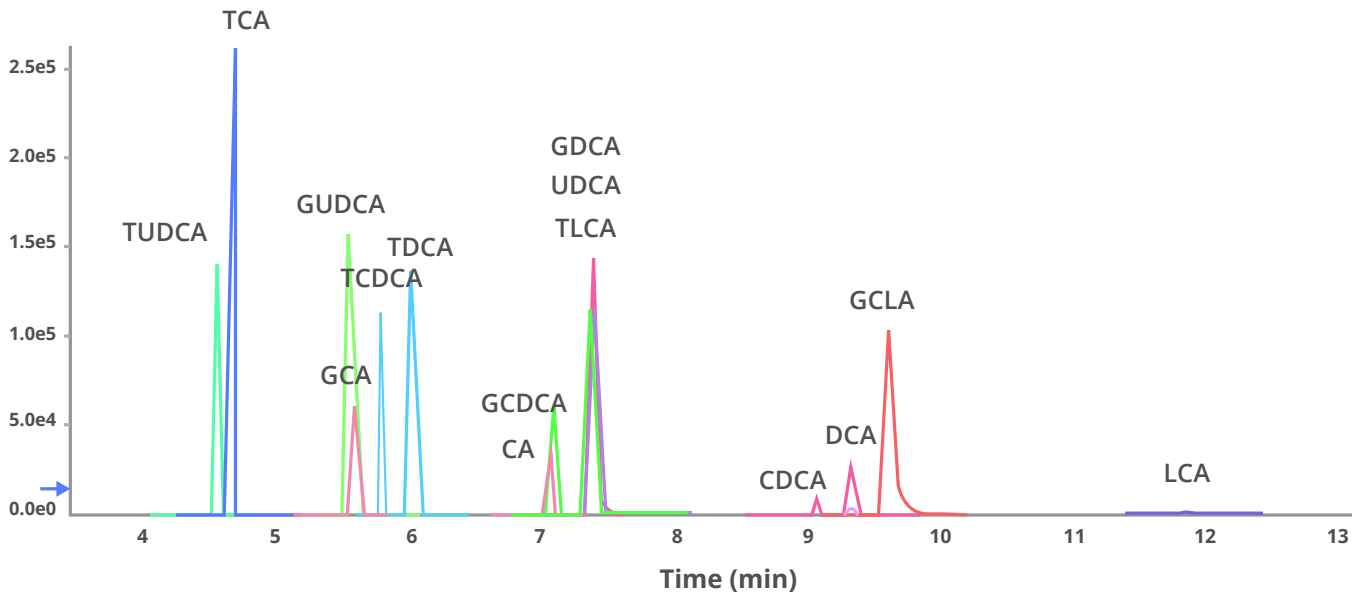
**Narrow XIC Window:** The high-resolution capabilities of the system allow for a narrow extracted ion chromatogram (XIC) window, which minimizes the inclusion of chemical background noise and interferences, enhancing the accuracy of quantitation in bile acid analysis.

**Potential for Faster Analysis:** The unique EAD spectra can provide diagnostic fragments that may reduce the need for long-duration chromatographic separation of isomers, potentially allowing for a more rapid assay with reduced run times.

# Method Development and Chromatographic Analysis

**The study conducted a detailed analysis of BAs using a method that required careful scheduling to reduce the duty cycle and improve sensitivity and quantitation.**

## Chromatographic resolution of bile acids is the key to success



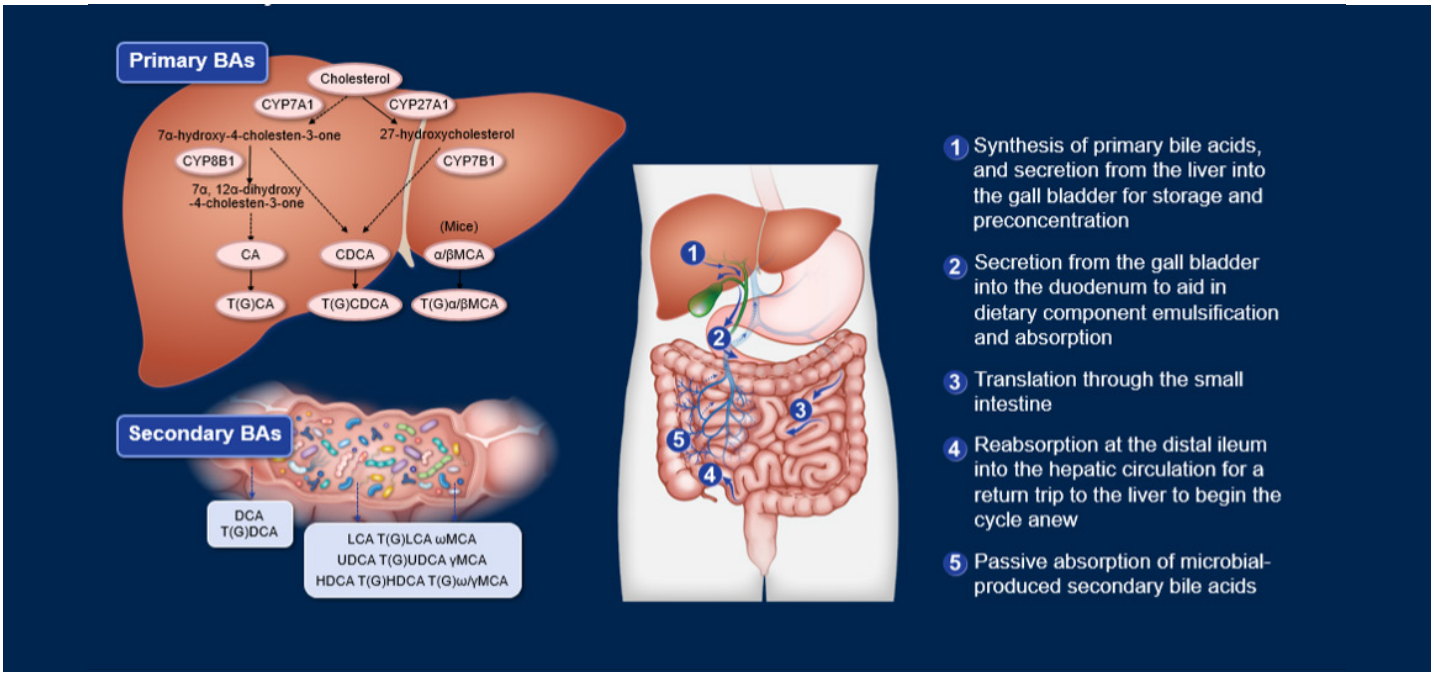

## Key Findings from the Study

- **Isobaric BA Pairs**
  The method differentiated between isobaric BAs, which have the same mass but different structures, through chromatographic resolution and mass spectrometry.
- **Set #1, Beta-muricholic (β-MCA) and Cholic Acid (CA)**
  The primary BAs β-MCA and CA share common precursor ions (m/z 407), but β-MCA possesses a distinct "diagnostic" product ion transition to m/z 371.
- **Set #2, Ursodeoxycholic acid (UDCA), Chenodeoxycholic Acid (CDCA), and Deoxycholic Acid**
  UDCA, CDCA, and DCA were all chromatographically resolved. In all instances, these metabolites have a common precursor ion (m/z 391), but DCA can be uniquely identified because of a distinct product ion transition to m/z 345.
- **Set #3, Glycoursodeoxycholic Acid (GUDCA), Glycochenodeoxycholic Acid (GCDCA), and Glycodeoxycholic Acid (GDCA)**
  All the glyco-conjugated BAs require chromatographic resolution because they share common MRM transitions of m/z 448 > m/z 74.
- **Set #4, Tauroursodeoxycholic Acid (TUDCA), Taurochenodeoxycholic Acid (TCDCA), and Taurodeoxycholic Acid (TDCA)**
  All the tauro-conjugated BAs require chromatographic resolution because they share common MRM transitions of m/z 498 > m/z 80.
- **Other BAs**
  Additional BAs analyzed included glycocholic acid (GCA), taurocholic acid (TCA), taurolithocholic acid (TLCA), glycolithocholic acid (GLCA), Alloisolithocholic acid (AILCA), and lithocholic acid (LCA).
- **Resolution and Quantitation**
  The method achieved baseline resolution for the separation of BA isobars, which allowed for accurate quantitation.
- **Internal Standards**
  The analysis provided a 1-to-1 parity between unlabeled analytical standards and deuterated internal standards.

## Bile Acid Synthesis and Biotransformation

BAs are created in the liver from cholesterol and include primary BAs such as cholic acid, chenodeoxycholic acid, and alpha-beta muricholic acid (produced predominantly in mice). These primary BAs can be further conjugated with the amino acids taurine and glycine in the liver to form tauro- and glycoconjugates. These synthesized BAs are then stored and concentrated in the gallbladder.

Upon eating, bile is released into the duodenum to help emulsify dietary fats and aid in their absorption. The bile then travels through the small intestine and is predominantly reabsorbed in the distal ileum, returning to the liver via the portal vein. A minor fraction (~5% of BAs) reaches the large intestine, where these compounds are modified by gut bacteria into secondary BAs.




# Case Studies

## Dried Fecal Spot Bioanalysis for BA Profiling in Microbiome Research

### Study summary

Dried matrix spots, a technique traditionally used in newborn screening, have been adapted for microbiome research to analyze fecal samples. This study aimed to assess the utility of a novel dried fecal spot (DFS)-based bioanalytical format for BA profiling, comparing the results with standard sample preparation methods.

**Figure 1:** 75µL dried blood spot on a Guthrie card

### Methodology

Stool samples were collected from healthy subjects, patients with diarrhea, and patients with *Clostridioides difficile (C. diff)* infections. Samples were processed to extract metabolites, spotted onto Capitainer B devices, dried, and shipped to the analysis site. Both DFS and residual liquid samples were analyzed using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). Data were analyzed with two-way ANOVA and post hoc tests to determine statistical significance.

### Key findings

BA profiles from DFS were nearly identical to those obtained from standard sample preparations. There were no significant differences in individual BA concentrations across the three cohorts when comparing DFS to standard methods.

The primary-to-secondary BA ratios were also consistent between DFS and standard preparations, with no significant differences observed. The study highlighted the convenience of using DFS for ambient temperature shipment and storage.

### Conclusions

DFS-based bioanalysis appears to be a reliable method for BA profiling in microbiome research, offering comparable results to traditional methods. The ambient temperature stability of DFS samples simplifies logistics and may lower costs. The research team plans to explore additional microbiome-relevant metabolites to further the utility of DFS in bioanalysis.

### Implications

The successful use of DFS for BA profiling suggests that this method could be widely adopted for microbiome studies, potentially leading to easier sample collection and transport, especially in large-scale or remote studies. This could facilitate more extensive microbiome research, with implications for understanding and treating various gastrointestinal diseases, including *C. diff* infections.

## BA Dysregulation Inintrahepatic Cholestasis of Pregnancy

### Study summary

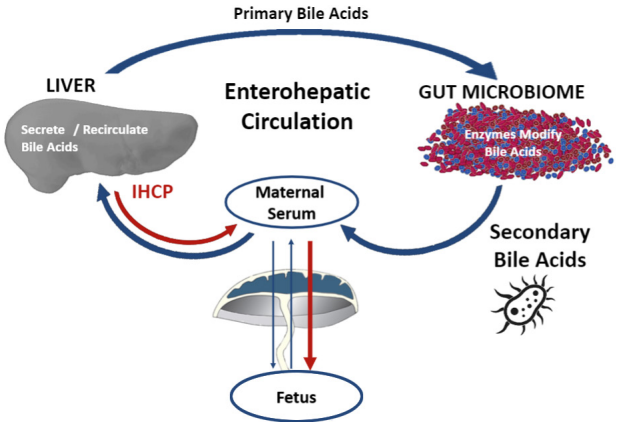
Intrahepatic Cholestasis of Pregnancy (IHCP) is a liver disorder characterized by impaired BA regulation, leading to elevated levels of circulating BAs. These increased BAs pose a risk to the fetus, potentially causing cardiotoxic effects and increasing the likelihood of stillbirth. While Ursodiol is used to alleviate maternal pruritus associated with IHCP, it does not reduce the risk of stillbirth. IHCP prevalence ranges from 0.3% to 2.5%, with higher rates in Hispanic populations and among women with gestational diabetes mellitus.

### Methodology

Maternal blood was collected using K2 EDTA, processed to plasma via centrifugation, and stored at -80°C in a biorepository. Upon thawing, a 50-microliter aliquot of plasma was mixed with 200 microliters of methanol, vortexed, and centrifuged to produce a clarified plasma extract. A 50-microliter sample of this extract was then combined with 450 microliters of an internal standard solution, vortexed, and placed into auto-sampler tubes for LC-MS/MS-based targeted bioanalysis.

### Key findings

The study aimed to identify concentration differences in individual BAs between healthy controls and IHCP patients. No statistical differences were observed for most BAs, but significant differences were found for specific BAs,




including GCDCA and TCDCA, and GCA and TCA, in IHCP patients. Total BA concentration was also significantly higher in IHCP patients. The study concluded that the elevated levels of certain BAs and total BAs in IHCP could potentially contribute to fetal cardiotoxicity.

### Future directions

The study suggests further investigation into the BAs identified as significantly elevated in IHCP patients to determine their role in fetal cardiotoxicity and to explore potential interventions that could mitigate the risks associated with IHCP.

## Microvillus Inclusion Disease and its Impact on BA Transport

### Study summary

Microvillus Inclusion Disease (MVID) is a rare and severe congenital disorder characterized by life-threatening diarrhea shortly after birth, which leads to a dependency on parenteral nutrition and potentially requires an intestinal transplant. Electron microscopy of intestinal enterocytes in MVID patients shows characteristic microvillus inclusions (see red ellipse on the electron micrograph).

### Pathophysiology

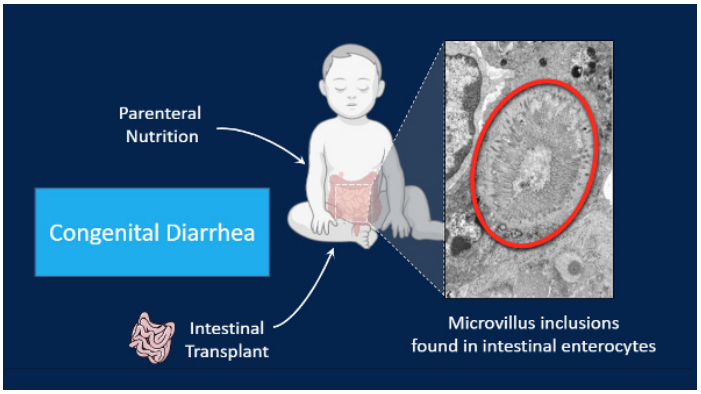
MVID is linked to mutations in the myosin 5B gene, which disrupt the normal trafficking of transporter proteins to the apical membrane of enterocytes. Myosin 5B normally interacts with F actin to deliver cargo, such as ion transporters, to the cell surface and is involved in the recycling of these transporters. Mutations in myosin 5B lead to the mislocalization of transporters and endocytic vesicles, contributing to the formation of microvillus inclusions and impaired nutrient absorption.

### Methodology

A myosin 5B knockout mouse model was developed to mimic human MVID. Mice displayed symptoms consistent with MVID, including high stool water content, decreased body weight, and early mortality. Tissues were harvested, homogenized with silica beads and methanol, and centrifuged, and the supernatant was analyzed using LC-MS.

### Key findings

The myosin 5B knockout mice showed significant elevation in ileal concentrations of primary BAs like CA and β-MCA when compared to littermate controls. Hepatic levels of primary BAs, including CA, DCA, and β-MCA, were significantly reduced. Conjugated primary (i.e., GCA and TCA) and secondary BAs (i.e., TCDCA and TUDCA) also showed decreased concentrations in the liver. Immunofluorescent staining revealed mislocalization of the apical sodium-dependent BA transporter (ASBT) in the ileum of myosin 5B knockout mice, indicating that ASBT localization is also affected by the mutation.




### Conclusions

The study confirms that the loss of myosin 5B in mice leads to microvillus inclusions similar to those observed in human MVID. There is an increase in ileal BAs and a decrease in hepatic BAs, which may be due to the mislocalization of BA transporters like ASBT within the enterocytes. These findings provide insight into the molecular mechanisms underlying MVID and its impact on BA homeostasis.

# Conclusion

Advancements in mass spectrometry, particularly with the introduction of the ZenoTOF 7600 system, have significantly improved the analysis of bile acids.

This has implications for a wide range of research areas, including disease diagnosis, understanding metabolic pathways, and the development of new treatments.

### Further Reading and Resources

- ZenoTOF 7600 system ››
- Quantitative analysis and structural characterization of bile acids using the ZenoTOF 7600 system ››
- Using targeted LC-MS/MS-based metabolomics to measure a broad constellation of bile acids/salts in disorders of human health ››
- Ruggedness testing of liquid chromatography-tandem mass spectrometry system components using microbiome-relevant methods and matrices ››
- A high-throughput LC-MS/MS method for the measurement of the bile acid/salt content in microbiome-derived sample sets ››
- Repurposing dried blood spot device technology to examine bile acid profiles in human dried fecal spot samples ››