Sensitive and selective quantitation of bile acids using targeted MS2/MS3 on the Stellar mass spectrometer

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Abstract

Purpose: Demonstrate the sensitive and selective quantification of bile acids and their conjugates using the hybrid quadrupole-linear ion trap Stellar MS in multiple scan modes.

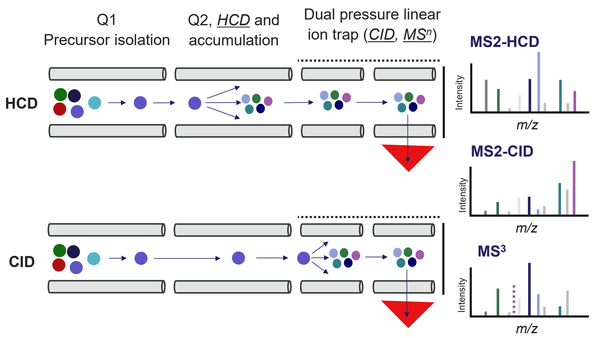
Methods: The bile acid compounds were separated with a 9-minute LC gradient and optimized on Stellar MS for simultaneous tMS2 and tMS3 acquisition. Optimal scan modes were selected for the quantification of bile acids in patient serum samples.

Results: Stellar MS provides alternative fragmentation modes to enhance sensitivity and selectivity for the accurate quantification of bile acids in human serum.

Introduction

Bile acids (BA), synthesized from cholesterol in the liver, are crucial for lipid digestion and absorption. In the gastrointestinal tract, primary bile acids are converted by host microbiota into various secondary and conjugated bile acids. Disordered microbiomes can alter the composition and concentration of the bile acid pool, impacting human health. Normally, blood levels of bile acids are low, but they can increase due to disorders such as liver disease. Consequently, identifying and quantifying these compounds has become a significant research focus. The Thermo Scientific™ Stellar™ mass spectrometer (MS) is a hybrid quadrupole, dual-pressure linear ion trap MS that enhances quantitative capabilities by combining two modes of fragmentation with rapid MS2 and MS3 scans in a single run (**Figure 1**).[1] We here present a highly selective targeted MS2/MS3 approach using the Stellar MS for the quantitation of bile acids and their conjugates in serum.

Figure 1. Schematic of PRM (tMSn) acquisition with Stellar MS.



Materials and methods

Sample preparation

Reference standards of bile acids and their labeled internal standards (IS) were purchased from Cambridge Isotope Laboratories. LC-MS grade water, methanol, acetonitrile, and formic acid were purchased from Fisher Scientific. A calibration curve was prepared in 50% methanol from 0.1 nM to 1000 nM. Serum samples were collected from children with and without autism spectrum disorder (ASD) and processed through Phenomenex Phree plates with 1% formic acid in acetonitrile. Samples were dried and reconstituted in 50 µL of 400 nM IS in 50% methanol. 2 µL was injected for LCMS analysis.

LC-MS parameters and data analysis

Samples were analyzed on a Thermo Scientific™ Vanquish™ Horizon UHPLC system coupled to a Stellar MS. Separation of the bile acids was achieved with a Waters Acquity BEH C18 column (2.1 x 100 mm, 1.7 µm) at 50 ° C. The LC gradient and extracted ion chromatograms of each bile acid are shown in Figure 2. Stellar MS parameters for both tMS2 and tMS3 acquisition were optimized for each compound using mzCloud™ libraries and experimental data of ramped collision energies (CE). The final method included 200+ qualitative and quantitative transitions with retention time windows of 0.8 min. Data were acquired using Thermo Scientific™ Xcalibur™ software version 4.7 and processed using Thermo Scientific™ TraceFinder™ software version 5.2.

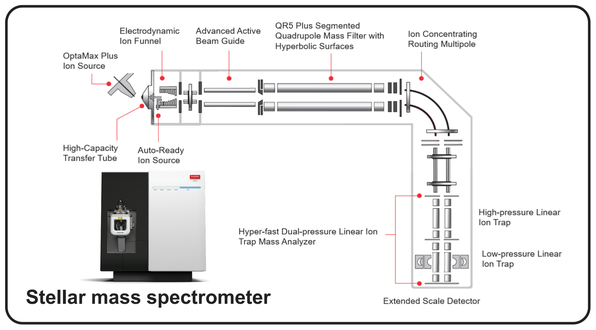
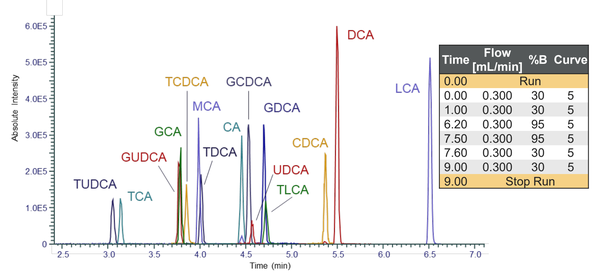


Figure 2. Representative EIC of the separation of all bile acid compounds. The inserted table shows the LC flow gradient over a 9-minute run time.



Time	Flow [mL/min]	%B	Curve
0.00	Run		
0.00	0.300	30	5
1.00	0.300	30	5
6.20	0.300	95	5
7.50	0.300	95	5
7.60	0.300	30	5
9.00	0.300	30	5
9.00	Stop Run		

Results

Compound optimization – MS2 and MS3 parameters

Although most bile acids share similar structures, employing alternative fragmentation modes can enhance their differentiation. When comparing HCD and CID for tMS2 acquisition, conjugated bile acids showed a preference for HCD fragmentation. This approach yielded better ion ratios, including fragments containing the base amino acid structures (**Figure 3**). Conversely, CID fragmentation predominantly produced fragment ions of the bile acid core.

Interferences and high baseline noise can create challenges for certain compounds, especially in complex matrices or near the limit of quantitation (LOQ). These cases benefit from utilizing multi-stage fragmentation (tMS3) to introduce new or enhance the signal of other diagnostic ions. **Figure 4** demonstrates this with the detection of cholic acid. Secondary fragmentation of the 343 *m/z* quantifier ion resulted in a cleaner baseline for peak integration, maintaining sufficient scans across the peak and reducing the LOQ by two calibration points compared to tMS2-CID acquisition.

Another key factor in determining the optimal scan mode for each bile acid compound is the dynamic range it covers. Using a calibration curve, we detected most bile acids over 4 orders of magnitude, from <1 nM to 1000 nM. **Figure 5A** shows example chromatograms across the curve for three bile acids: MCA, DCA, and LCA. The linearity of the curve is demonstrated by DCA, with R^2 = 0.9989 down to 0.5 nM (**Figure 5B**).

Figure 3. Comparison of fragmentation patterns with CID and HCD activation in GCDCA (A) and TUDCA (B). The proposed fragmentation mechanisms for the quantitative ions (denoted by *) of GCDCA (C) and TUDCA (D).

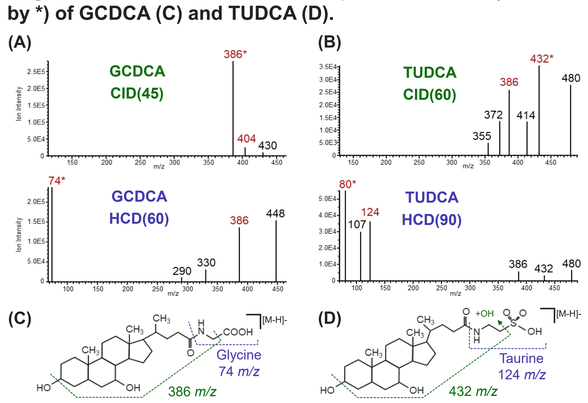


Figure 4. Baseline noise reduction with tMS3. (A) The quantification of cholic acid with *m/z* 343 in tMS2-CID exhibiting low signal-to-noise levels near LOQ. (B) Improved sensitivity and selectivity using tMS3 acquisition with comparable Quan-Qual ion ratios to tMS2.

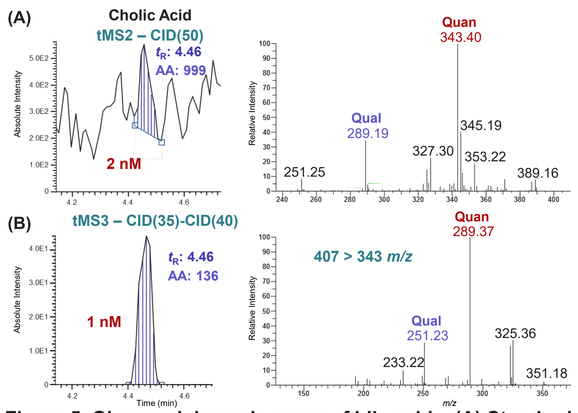


Figure 5. Observed dynamic range of bile acids. (A) Standard curve chromatograms of MCA, DCA, and LCA from 2 fmol to 2pmol on-column. (B) Standard curve for DCA in neat solution, 0.5 to 1000 nM.

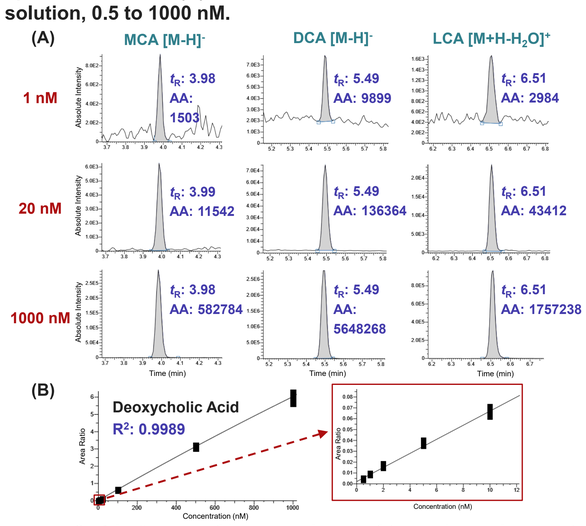
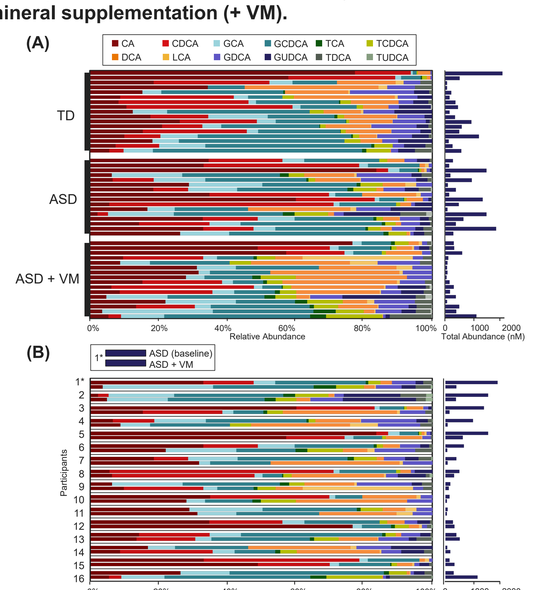


Figure 6. Bile Acid profiles in children of typical development (TD) and with autism spectrum disorder (ASD). (A) Relative abundance of 12 quantified bile acids for each sample (TD = 17, ASD = 16, ASD+VM = 16). The right panel shows the total bile acid abundance (in nM) per sample. (B) Profiles of participants with ASD before (baseline) and after vitamin-mineral supplementation (+ VM).



Quantification of bile acids in human serum

The optimization of Stellar MS tMS2/tMS3 capabilities enables accurate and robust quantification of bile acids in human serum, even at low levels. **Figure 6** shows the relative abundance of 12 bile acids across 49 serum samples from typically developing (TD) children and children with autism spectrum disorder (ASD) before and after vitamin-mineral supplement treatment (ASD + VM). Total bile acid abundance ranged from 5 nM to 2100 nM. Comparison revealed that 11 of 16 participants had a >30% decrease in total bile acids post-treatment, matching trends observed in the absolute abundance of primary and primary conjugated bile acids – CDCA, GCA, GCDCA, TCDCA.

Table 1 summarizes the selected scan mode for each bile acid, LOQ in pg on-column, and the %RSD of the integrated internal standard response across all study samples (N = 237). LOQ was determined using a weighting factor of 1/x with R^2 values greater than 0.99, |%Diff| < 30%, and %RSD < 20% over 6 technical replicates. Peak areas of the 13 internal standards were robust for sample analysis with most compounds under 8% RSD and all below 15% RSD.

Table 1. The LOQ of each bile acid – converted to pg on-column – with the optimal scan mode and collision energy. The reproducibility of this method is represented by the internal standard response %RSD over 237 serum samples.

Bile Acid	Abbrev.	Scan mode (CE)	LOQ (pg)	Sample IS %RSD
Cholic acid	CA	CID(35)-CID(40)	0.82	--
Deoxycholic acid	DCA	CID(50)	0.39	6.7%
Glycocholic acid	GCA	HCD(60)	0.93	7.6%
Glycochenodeoxycholic acid	GCDCA	HCD(60)	0.90	7.2%
Glycodeoxycholic acid	GDCA	HCD(60)	0.90	6.6%
Glycoursodeoxycholic acid	GUDCA	HCD(60)	--	6.5%
Lithocholic acid	LCA	HCD(40)	0.38	14.6%
Muricholic acid	MCA	CID(45)	1.63	7.5%
Taurocholic acid	TCA	HCD(90)	1.03	6.7%
Taurolithocholic acid	TLCA	CID(55)	1.93	--
Taurochenodeoxycholic acid	TCDCA	HCD(90)	--	6.7%
Taurodeoxycholic acid	TDCA	HCD(90)	2.00	6.3%
Tauroursodeoxycholic acid	TUDCA	HCD(90)	1.00	6.4%
Ursodeoxycholic acid	UDCA	CID(45)	15.69	12.9%

Conclusions

Targeted MS2/MS3 acquisition using Stellar MS enables sensitive and selective quantification of bile acids and their conjugates in human serum. By employing alternative fragmentation techniques, we improved detection and enhanced ion ratios for more accurate quantification at lower concentrations. This method was successfully applied to a cohort study of children with ASD, identifying and quantifying liver-synthesized bile acids in serum samples. Future work will focus on expanding the method to include additional bile acid conjugates, providing a more comprehensive profile of bile acid metabolism.

References

1.Remes, P. M., et. al., J. Proteome Res. 2024, 5476.

Acknowledgements

We gratefully thank all individuals with ASD and their families for participating in the study and Joel Meraz for laboratory assistance at ASU.

General Laboratory Equipment – Not For Diagnostic Procedures.

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