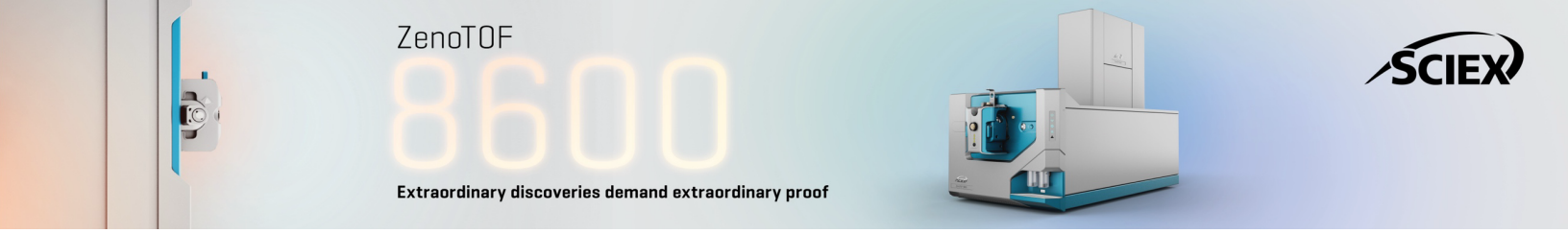

Quantitative and qualitative bile acid analysis on the ZenoTOF 8600 system using EAD

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This technical note demonstrates the power and capability of the ZenoTOF 8600 system to sensitively detect, quantify, and structurally characterize the bile acid content in human plasma.

The analysis of bile acids in human samples has become increasingly important as insights into the prominent biological role of this class of molecules are discovered [1,2]. Analysis of bile acids by triple-quadrupole MS (TQMS) systems is challenging because of the high chemical background found in several precursor ions to precursor ion-based multiple-reaction monitoring (MRM) transitions used in current state-of-the-art assays (1-4). High-resolution mass spectrometry
(HRMS) enables the extraction of fragment ions with a narrow mass-to-charge (*m/z*) window, which can reduce background chemical interferences and improve the signal-to-noise (S/N) of the assay as previously demonstrated [3].

The detection of individual bile acid isomers currently depends on chromatographic resolution because collision-induced dissociation (CID)-based fragmentation cannot distinguish these isomeric metabolites. However, a novel fragmentation method, electron-activated dissociation (EAD) can generate unique fragments, even among isomers that may help reduce the time required for analysis.

Here, the ZenoTOF 8600 system was used to quantify the bile acid content of human plasma sample extracts using both CID- and EAD-based fragmentation methods. EAD-based fragmentation was strategically used when isomeric pairs overlapped to provide specificity and enable faster analysis times.

Key features of bile acid analysis on the ZenoTOF 8600 system

- The ZenoTOF 8600 system can detect and quantify bile acids with an approximately 12-fold better sensitivity than the ZenoTOF 7600 system
- The narrow fragment ion extraction window possible with HRMS improves assay sensitivity by reducing background chemical interferences and, consequently, increases the S/N of the assay
- EAD can generate diagnostic fragment ions to enable the distinction between bile acid isomers, which improves the overall specificity and speed of the analysis

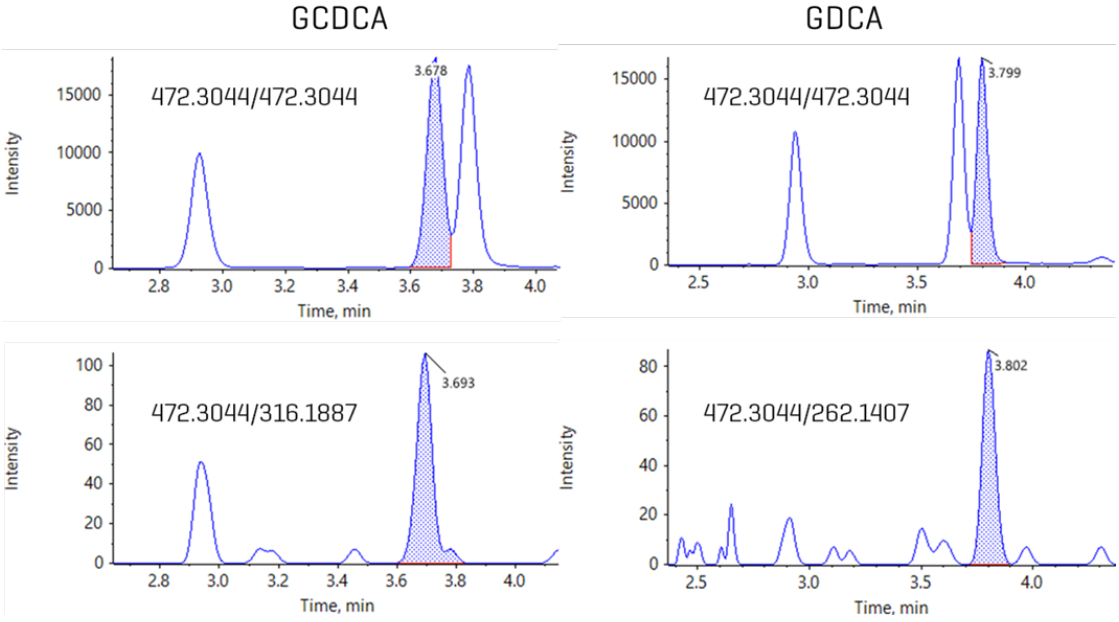

Figure 1. Detection and quantitation of bile acid isomers in human plasma using EAD. Using a fast, 10-minute gradient, bile acids were analyzed on the ZenoTOF 8600 system, Isomers such as GCDCA and GDCA are indistinguishable using CID-based fragmentation. Using EAD, unique, diagnostic fragments were enabled their accurate quantitation despite the chromatographic overlap. The upper panels show the two isomers overlapping when using a ; the lower panels show the ability to isolate each isomer separately when a unique, EAD-based fragment is used.

Introduction

Bile acids are amphipathic steroidal molecules synthesized in the liver from cholesterol and play a crucial role in the digestion and absorption of dietary lipids [1]. Primary bile acids, such as cholic acid and chenodeoxycholic acid, are conjugated with glycine or taurine before being secreted into bile. Once released into the intestinal lumen, these acids undergo microbial transformation to form secondary bile acids like deoxycholic acid and lithocholic acid that are collectively termed microbially conjugated bile acids (MCBAs). Enterohepatic circulation maintains a dynamic balance of bile acid synthesis, secretion, reabsorption, and excretion. Beyond their digestive functions, bile acids are now recognized as signaling molecules involved in metabolic regulation and immune responses, acting through nuclear receptors such as FXR and membrane-bound G-protein coupled receptors [2].

Mass spectrometry (MS) has become the analytical method of choice for the quantitation and profiling of bile acids due to its sensitivity, specificity, and ability to differentiate structurally similar compounds. [3-5] The use of HPLC ESI-MS/MS allows for the simultaneous detection of multiple bile acid species, including conjugated and unconjugated forms, in biological matrices such as plasma, bile, and feces. Targeted MS approaches using multiple reaction monitoring (MRM) provide high-throughput and quantitative data essential for clinical and research applications in liver disease, metabolic disorders, and gut microbiome studies.

Recently, we reported the analysis of bile acids using the ZenoTOF 7600 system, which is a hybrid quadrupole time of flight instrument (QTOF) that measures compounds with high resolution (~35K) and mass accuracy (<2 ppm) [TN ref]. In the negative ion mode, bile acid analysis by nominal mass instruments is challenging due to solvent-based high chemical background noise. With accurate mass systems, this issue can be mitigated using narrow product ion extraction windows (XIC), which minimize noise and enable a similar calculated LOQ for the ZenoTOF 7600 system as with the highly sensitive triple quadrupole SCIEX 7500 system. Using the ZenoTOF 8600 system, these studies were extended to capitalize on the instrument's improved sensitivity.

The ZenoTOF 8600 system has unique design features that make it particularly suited for targeted bile acid analysis. First, the instrument has a complementary fragmentation mode, EAD, that provides unparalleled structural characterization of small molecules [6-10]. Second, improved hardware, such as the front end with the Optiflow Pro source, a larger instrument orifice, and an advanced optical detector, enables higher sensitivity compared to the ZenoTOF 7600 system. Third, the instrument's front end is equipped with a DJET+ with Mass Guard, which has been shown to reduce instrument contamination on the SCIX 7500+ system (11), an important consideration for lipid and metabolite analysis, especially in the

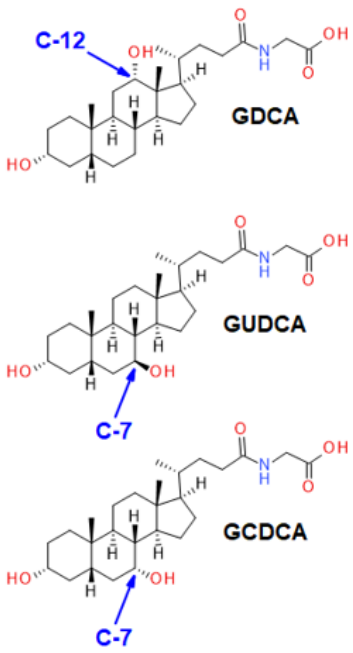

Figure 2. The glucodeoxycholic acid subclass of bile acids. Bile acids are cholesterol-derived amphipathic molecules of saturated hydroxylated C-24 sterols. GDCA has a hydroxyl moiety at carbon 12 (C-12), whereas GUDCA and GCDCA are hydroxylated at carbon 7 (C-7). The latter two isomers are characterized by a different stereochemistry at the number 7 carbon. Quantitative specificity for this class of molecules is highly dependent on chromatographic resolution when analyzed using CID-based fragmentation.

complex matrices common to bile acid analysis, including plasma, feces, and bile.

Bile acids are cholesterol-derived amphipathic molecules of saturated hydroxylated C-24 sterols. As shown in **Figure 2**, the location and stereochemistry of the hydroxyl functional groups define the different isomers within each sub-class. CID-based fragmentation of bile acids primarily yields product ions related to the bile acid head group, and relatively few fragments are generated from the ring structure; hence, CID alone does not generate diagnostically useful fragment ions for identification or quantitation purposes. Using chromatography to resolve bile acid isomers requires relatively long chromatographic gradients, which are not conducive to high-throughput analysis. An alternative to lengthy chromatographic gradients and CID-based fragmentation is EAD. EAD-based fragmentation provides ample fragments derived from the sterol ring structure, and it can distinguish the isomers of each bile acid subclass without extensive chromatographic method development.

In this technical note, the speed and sensitivity of the ZenoTOF 8600 system were leveraged to quantify bile acids in prepared plasma samples. To demonstrate the power of EAD to improve structural specificity during quantitative analysis, unique EAD-based fragments were identified for potentially co-eluting isomer pairs and used strategically in conjunction with CID-based fragmentation to enable a

faster assay while maintaining quantitative specificity, accuracy, and precision.

Methods

Materials: Bile acid standard mixtures were purchased from Cambridge Isotopes Laboratories. The stable isotope-labeled bile acid mixes (unconjugated, cat# MSK-BA1; conjugated, MSK-BA2) were used as internal standards, and unlabeled bile acid mixes (unconjugated, cat# MSK-BA1-US; conjugated, MSK-BA2-US) were used as primary reference standards. All solvents were of LC-MS grade and obtained from Burdick and Jackson and Fisher Scientific.

Sample preparation: Human blood (research use only) was collected into tubes containing potassium ethylenediamine tetraacetic acid (K_2EDTA) anti-coagulant following the procedure approved by the institutional review board (IRB) of BCM. The plasma was isolated from the red blood cell fraction by centrifugation. A 50 µL volume of each plasma sample was pipetted into individual 0.6 mL Eppendorf tubes, and a 200 µL volume of methanol was added to precipitate plasma proteins (primary DF=5-fold). All samples were vortex-mixed for 30 seconds and centrifuged at 17,000*g* for 5 minutes, and the supernatant for each clarified plasma extract sample was transferred into fresh vials. A 50 µL volume of each clarified plasma extract sample was transferred into fresh glass vials. A 450 µL volume of an internal standard solution (10 nM deuterated BAs) was added to each sample vial (DF=50-fold overall), and the vials were capped and vortex-mixed for 30 seconds. And prepared for analysis by HPLC ESI-MS/MS.

Chromatography: Extracted metabolites were resolved using a Nexera 40 Series UHPLC system equipped with a Restek Raptor C18 column (100 x 2.1 mm; 5.0 µm particle size). The autosampler sample bay was maintained at 10 °C , and a 5 µL sample volume was injected on column. The column oven temperature was kept at 50°C with a constant mobile phase flow rate of 0.3 mL/min for a total run time of 17 min. The mobile phase compositions were (A) 10 mM ammonium formate in water and (B) pure acetonitrile. Gradient details are shown in **Table 1.** Alternatively, the gradient was altered to enable a 10 min total run time (**Table 2**) in which EAD-based fragmentation was selectively employed to maintain compound specificity for co-eluting isomers that are not distinguishable using CID-based fragmentation, an example of which is shown in **Figure 1**.

Table 1. Chromatographic gradient conditions

Time (min)	Mobile phase A (%)	Mobile Phase B (%)
0	75	25
1.5	75	25
13	20	80
14.5	20	80
14.6	75	25
17	75	25

Table 2. 10-minute chromatographic gradient conditions

Time (min)	Mobile phase A (%)	Mobile Phase B (%)
0	75	25
1.5	75	25
6	20	80
7.5	20	80
7.6	75	25
10	75	25

Mass spectrometry: Sample extracts were analyzed using the ZenoTOF 8600 system with an OptiFlow ProTurbo V ion source and a scheduled, high-resolution multiple reaction monitoring (sMRMHR) scan mode. CID-based fragmentation was used for the initial assessment of the system's performance using the 17-minute gradient. This experiment was repeated using EAD-based fragmentation. To speed up the experiment and reduce the total time to 10 min, an sMRMHr experiment was performed using mixed-mode fragmentation. For isomers that overlapped when the gradient was shortened, an EAD-based fragment that is diagnostic for the overlapping isomers was used. Otherwise, CID was used for analysis due to its greater sensitivity and shorter accumulation times. MS instrument parameters are presented in **Table 3**, and a comprehensive list of the compounds studied is shown in **Table 4**.

Table 3. Instrument parameter settings

Parameter	ZenoTOF 8600 system
Curtain gas (CUR)	35
Ion source gas 1 (GS1)	60
Ion source gas 2 (GS2)	80
CAD Gas (CAD)	7
Source temperature (TEM)	250 °C
Ion spray voltage (IS)	-4500 V/+5000V (CID/ EAD)
Declustering Potential (DP)	-80 V/+80V (CID/EAD)
CID Accumulation/dwell time	Variable (sMRM^{HR})
CID Collision energy (CE)	Molecule- dependent
Q1/Q3 Mass Resolution	N/A
EAD Accumulation/dwell time	40 ms
CE for TOF MS	-10 V/+10V (CID/EAD)
TOF MS mass range	70-1000 Da
TOF MS/MS mass range	40-400 Da
Time bins to sum	6
EAD Collision energy (CE)	12 V
Electron kinetic energy (KE)	13 eV
Zeno pulsing for MS/MS	Yes
Zeno threshold	20000

Table 4. Bile acid compound parameters

Bile Acid	Abbreviation	[M – H]-	$[M - H]^-$ Product ion	$[M + Na]^+$	Coliision energy (V)
Lithocholic Acid	LCA	375.29	375.29	399.288	-5
D4-Lithocholic Acid	D4-LCA	379.316	379.316	403.313	-5
Ursodeoxycholic Acid	UDCA	391.285	391.285	415.283	-5
D4-Ursodeoxycholic Acid	D4-UDCA	395.31	395.31	419.308	-5
Chenodeoxycholic Acid	CDCA	391.285	391.285	415.283	-5
D4-Chenodeoxycholic Acid	D4-CDCA	395.31	395.31	419.308	-5
Deoxycholic Acid	DCA	391.285	391.285	415.283	-49
D4-Deoxycholic Acid	D4-DCA	395.31	395.31	419.308	-49
Cholic Acid	CA	407.28	343.266	431.278	-49
D4-Cholic Acid	D4-CA	411.305	411.305	435.303	-49
Glycolithocholic Acid	GLCA	432.312	388.324	456.309	-48
D4-Glycolithocholic Acid	D4-GLCA	436.337	392.349	460.335	-48
Glycoursodeoxycholic Acid	GUDCA	448.307	74.025	472.304	-82
D4-Glycoursodeoxycholic Acid	D4-GUDCA	452.332	74.025	476.329	-82
Glycochenodeoxycholic Acid	GCDCA	448.307	74.025	472.304	-82
D4-Glycochenodeoxycholic Acid	D4-GCDCA	452.332	74.025	476.329	-82
Glycodeoxycholic Acid	GDCA	448.307	74.025	472.304	-82
D4-Glycodeoxycholic Acid	D4-GDCA	452.332	74.025	476.329	-82
Glycocholic Acid	GCA	464.302	402.302	488.299	-48
D4-Glycocholic Acid	D4-GCA	468.327	406.331	492.324	-48
Taurolithocholic Acid	TLCA	482.295	79.958	506.292	-137
D4-Taurolithocholic Acid	D4-TLCA	486.32	79.958	510.317	-137
Tauroursodeoxycholic Acid	TUDCA	498.289	79.958	522.287	-142
D4-Tauroursodeoxycholic Acid	D4-TUDCA	502.315	79.958	526.312	-142
Taurochenodeoxycholic Acid	TCDCA	498.289	79.958	522.287	-142
D4-Taurochenodeoxycholic Acid	D4-TCDCA	502.315	79.958	526.312	-142
Taurodeoxycholic Acid	TDCA	498.289	79.958	522.287	-142
D4-Taurodeoxycholic Acid	D4-TDCA	502.315	79.958	526.312	-142
Taurocholic Acid	TCA	514.284	124.008	538.282	-66
D4-Taurocholic Acid	D4-TCA	518.31	124.007	542.307	-66

During sMRMHr analysis, a full product ion spectrum is acquired. To identify diagnostic, EAD-based fragments for co-eluting bile acid isomers such as GCDCA and GDCA,

The Analytics module within SCIEX OS software was used for quantitation, and the Explore module was used to structurally characterize bile acids from EAD-based MS/MS spectra. The limit of quantitation (LOQ) was determined from the lowest standard injected with the %CV value < 15 and an accuracy within 85% and 115% of the expected values for the internal standard curve.

Results and Discussion

A bile acid standard curve was generated neat in solvent and analyzed on the ZenoTOF 8600 system. The parameter settings were optimized, as detailed in **Tables 3 and 4**, and the chromatographic gradient conditions resulted in the separation of each isomeric bile acid with baseline resolution **(Figure 3)**. The concentrations within the internal standard curve ranged from 0.011 to 100 nM, covering a dynamic range of > 4 orders of magnitude. An example curve acquired for gylcoursodeoxycholic acid (GUDCA) is presented in **Figure 4 (panel A)**, and the individual chromatograms for each injection of the standard curve from 0.011 to 50 nM GUDCA are shown (**panel B**). The associated quantitative statistics for GUDCA quantitation are shown in **Table 5.** The data derived from the internal standard curves were used to calculate the LOQ of each bile acid measured with the ZenoTOF 8600 system (**Table 6**). These values were determined as the lowest concentration standard injected that gave a %CV value <15% and an accuracy within the range of 85% to 115% of the expected value.

The LOQ values for the ZenoTOF 7600 system were calculated in a different way [BA TN] than they were in this report (see above). Additionally, on the ZenoTOF 7600 system, a 15 µL sample was injected, whereas only 5 µL was injected onto the ZenoTOF 8600

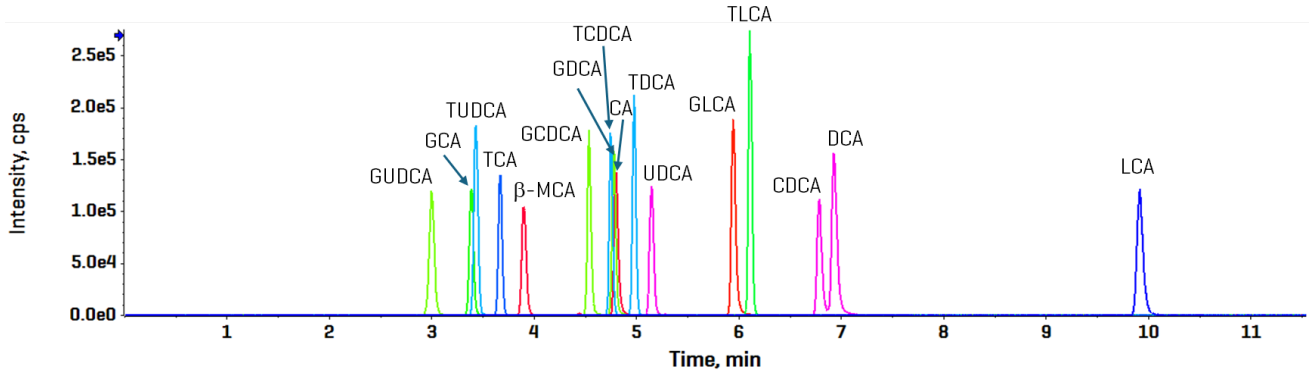

Figure 3. Elution profile of targeted bile acids using CID-based fragmentation in the negative ion mode. The 50 nM standard was injecte3d and analyzed using the sMRMHr scan mode. Total run time was 17 min.

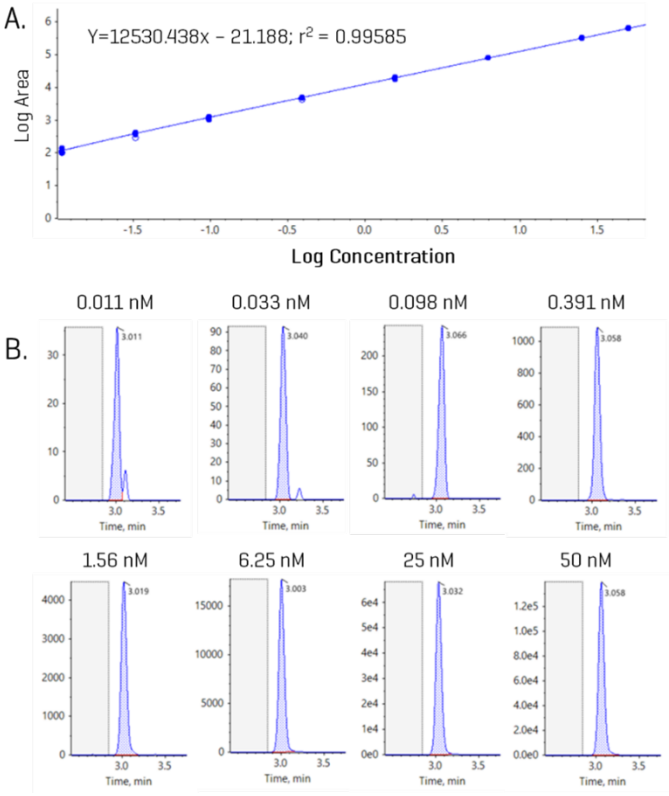

Figure 4. Example internal standard curve with representative peaks for GUDCA. **(A)** Internal standard curve for GUDCA from 0.011 to 50 nM. **(B)** Example chromatograms at each concentration level of the curve; blank region highlighted in grey.

system. To compare the relative sensitivity of the two different systems, the on-column injection amount at the LOQ was used as a reference (**Table 7**). The data show that the ZenoTOF 8600 system is an average of ~20-fold more sensitive than the ZenoTOF 7600 system (range = 1.1 to 61-fold improvement). The wide range may be, in part, due to the difference in the sources used with the two instruments. The ZenoTOF 7600 system is equipped with the Turbo V twin spray source, which optimizes differently and may have a different ionization capacity, or a differential ionization efficiency compared to the Optiflow Pro Turbo V ion source on the ZenoTOF 8600 system. The ion path of the ZenoTOF 8600 system is also different from the previous version, which may also slightly affect transmission in a compound-dependent manner.

The assay developed using neat standards was used to measure endogenous bile acids in human plasma. **Figure 5** shows the combined XIC data for all targeted bile acids measured with the sMRMHr workflow in human plasma extract. Except for lithocholic acid (LCA), all bile acids were detected and quantified using the ZenoTOF 8600 system. The data shown here were collected using the CID-based fragmentation mode, and the LC gradient extended over 17 minutes. The concentrations of bile acids detected in two representative plasma samples are shown in **Table 8**. The same experiment was performed using EAD-based fragmentation for all compounds (not shown), and those data were used to identify diagnostic fragment ions for bile acid isomers that co-elute using the faster, 10 min gradient.

Table 5. Quantitative statistics for GUDCA

Standard	*Standard concentration (nM)*	*Calculated concentration (nM)*	*Std dev*	*%CV*	*Average accuracy (n = 5)*
GUDCA	*0.011*	*0.011*	*0.0014*	***12.9***	***100.4***
GUDCA	*0.033*	*0.033*	*0.0017*	***5.13***	***101.0***
GUDCA	*0.098*	*0.093*	*0.0074*	***7.90***	***95.0***
GUDCA	*0.391*	*0.385*	*0.0087*	***2.27***	***98.5***
GUDCA	*1.56*	*1.57*	*0.0873*	***5.58***	***100.3***
GUDCA	*6.25*	*6.39*	*0.1008*	***1.58***	***102.3***
GUDCA	*25*	*26*	*0.6598*	***2.59***	***102.0***
GUDCA	*50*	*50*	*1.3860*	***2.76***	***100.5***

Table 6. LOQ values for bile acids on the ZenoTOF 8600 system

Bile acid	LOQ (nM)
LCA	0.098
UDCA	0.098
CDCA	0.033
DCA	0.033
CA	0.033
GLCA	0.033
GUDCA	0.011
GCDCA	0.011
GDCA	0.011
GCA	0.098
TLCA	0.033
TUDCA	0.011
TCDCA	0.033
TDCA	0.033
TCA	0.033

Table 7. Comparison of on-column injection load at LOD between the ZenoTOF 7600 and 8600 systems

Bile Acid	fmol on-column injection at LOQ		Fold-increase in sensitivity
	ZenoTOF 7600 system	ZenoTOF 8600 system	
LCA	6.075	0.490	12.4
UDCA	2.160	0.490	4.4
CDCA	0.446	0.165	2.7
DCA	0.188	0.165	1.1
CA	9.195	0.165	55.7
GLCA	10.035	0.165	60.8
GUDCA	2.025	0.055	36.8
GCDCA	1.277	0.055	23.2
GDCA	0.777	0.055	14.1
GCA	3.150	0.490	6.4
TLCA	3.165	0.165	19.2
TUDCA	1.115	0.055	20.3
TCDCA	4.635	0.165	28.1
TDCA	1.515	0.165	9.2
TCA	3.810	0.165	23.1

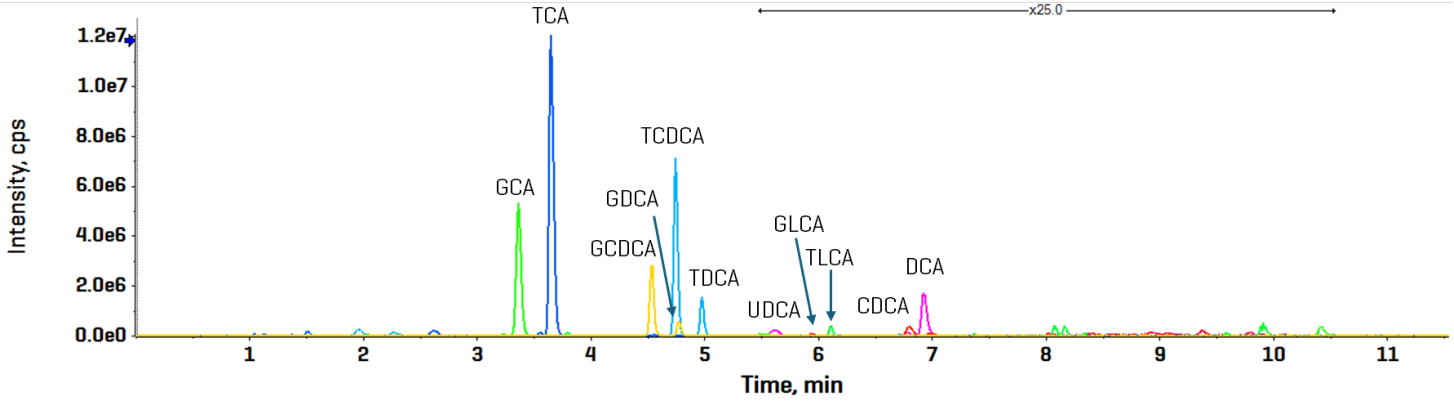

Figure 5. Bile acid detection and quantitation in human plasma using sMRMHr analysis on the ZenoTOF 8600 system

Table 8. Example quantitative results from 2 different human plasma samples

Bile Acid	Abbreviation	Sample 1		Sample 2	
		Concentration (nM)	%CV (n=3)	Concentration (nM)	%CV (n=3)
Lithocholic Acid	LCA	**ND**	N/A	**ND**	N/A
Ursodeoxycholic Acid	UDCA	**0.714**	4.9	**0.490**	4.3
Chenodeoxycholic Acid	CDCA	**12.4**	1.7	**0.612**	1.1
Deoxycholic Acid	DCA	**12.3**	2.4	**0.708**	6.0
Cholic Acid	CA	**6.03**	2.4	**5.32**	7.2
Glycolithocholic Acid	GLCA	**0.395**	6.0	**0.041**	14
Glycoursodeoxycholic Acid	GUDCA	**0.181**	1.0	**2.48**	1.0
Glycochenodeoxycholic Acid	GCDCA	**797**	1.4	**112**	3.7
Glycodeoxycholic Acid	GDCA	**146**	3.8	**0.863**	6.1
Glycocholic Acid	GA	**1570**	2.7	**206**	0.9
Taurolithocholic Acid	TLCA	**1.13**	0.89	**0.093**	3.7
Tauroursodeoxycholic Acid	TUDCA	**2.41**	4.3	**1.53**	4.1
Taurochenodeoxycholic Acid	TCDCA	**1510**	0.37	**344**	6.0
Taurodeoxycholic Acid	TDCA	**246**	2.5	**254**	0.30
Taurocholic Acid	TCA	**3780**	1.2	**313**	3.7

The EAD-based fragmentation functionality of the ZenoTOF 7600 system can provide significantly more structural details than that generated by CID-based fragmentation. Previously, we reported the identification of unique fragments for the bile acid isomers CDCA and

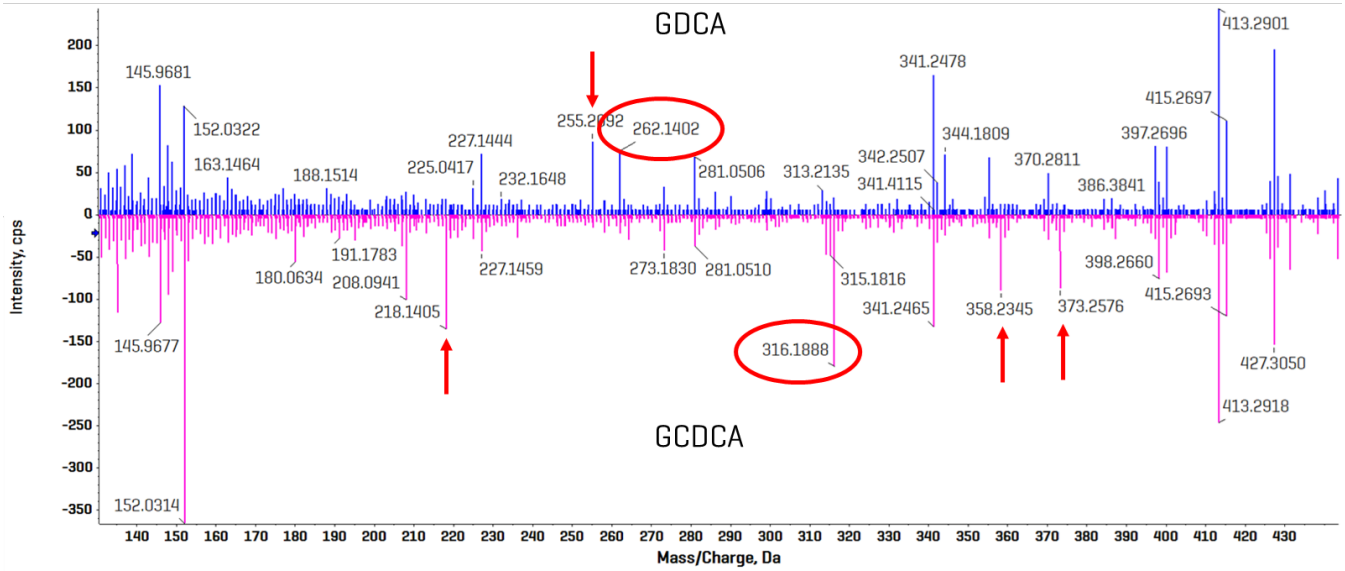

Figure 6. Comparison of EAD-based fragmentation of GDCA and GCDCA. A portion of the product ion spectra for GCDCA and GDCA is highlighted, with the spectrum for GDCA overlaid and inverted. Multiple fragments were identified for each bile acid isomer that appear to be unique and could serve as a selective fragment ion during analysis (red arrows). The fragment ions with *m/z* 262.1403 and 316.1888 were chosen for GDCA and GCDCA, respectively, due to their relatively high abundances.

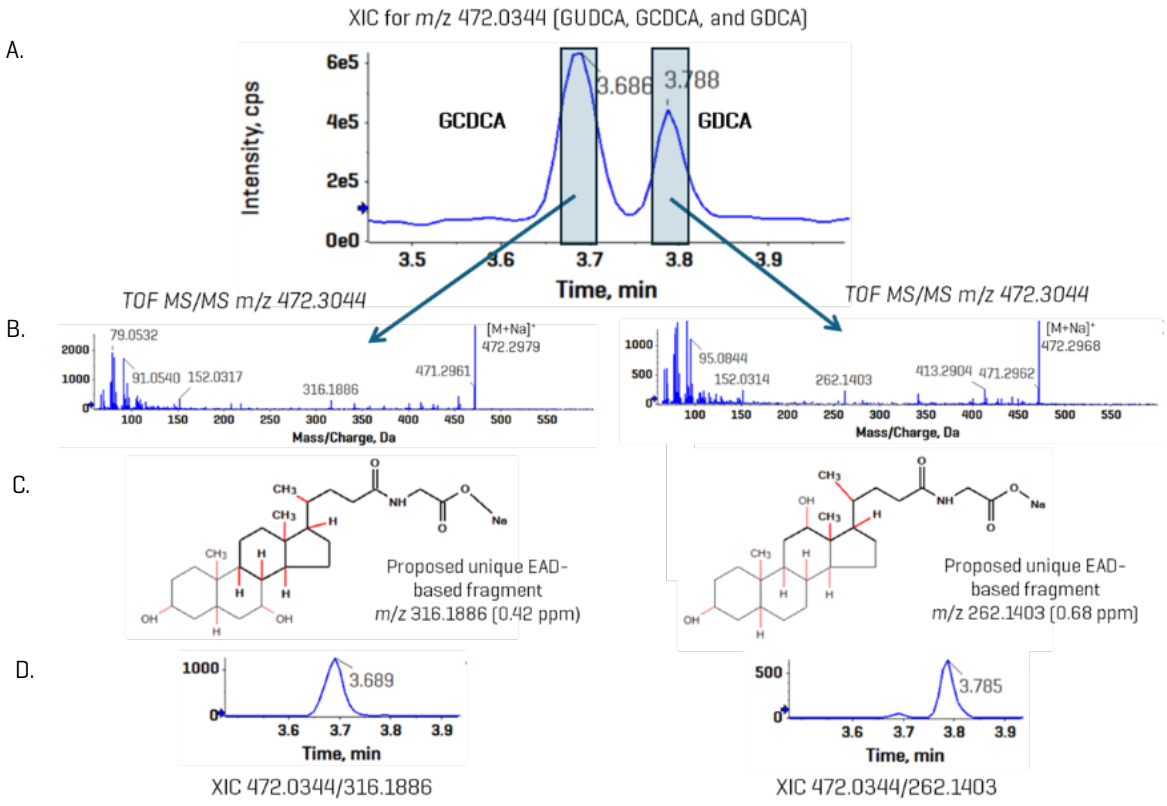

Figure 7. Resolution of bile acid isomers using EAD-based fragmentation. GCDCA and GDCA are bile acid isomers that have identical CID-based MS/MS spectra. Using EAD, unique, diagnostic fragments were observed for each isomer (A and B) in a standard mixture (6.56 nM). Using the fragments pane in SCIEX OS, structures for each fragment, *m/z* 316.1886 and 262.1403, were proposed for GUDCA and GDCA, respectively (C). Using a 10 mDa XIC window, panel D shows the improved specificity of the analysis for the two standards.

DCA [TN BA]. These isomers elute very closely and are responsible, in part, for the relatively long analysis time so that they remain chromatographically resolved. It was hypothesized that using EAD-based fragmentation for these isomer pairs would allow for a truncated chromatographic gradient and enable faster sample analysis. Here, we extended these findings using the ZenoTOF 8600

system and leveraged the ~10-fold increase in sensitivity to strategically use EAD-based fragmentation for specific bile acids during sMRMHr analysis. **Figure 6** shows a region of the EAD-derived product ion spectrum for GCDCA and GDCA acquired as neat standards; the spectrum for GCDCA is inverted so the differences in the spectra are more apparent. Fragment ions at *m/z* 262.1403 and 316.1888 were identified as unique, diagnostic ions for GDCA and GCDCA, respectively. Other apparently unique fragment ions for each bile acid isomer are indicated with red arrows. Having multiple unique fragment ions may help with quantitation, as fragment intensities can be added during quantitative processing in the Analytics module of SCIEX OS software.

Using the faster, 10-min gradient, the bile acid standards were analyzed using EAD-based fragmentation. **Figure 7, panel A,** shows the elution profile of GCDCA and GDCA using neat standards, with the peak-to-peak retention time difference of ~12 s. The product ion spectra are shown in **panel B**, with the diagnostic ion for each apparent in its respective spectrum. The fragment panel app within the SCIEX OS Explore module proposed 2 possible structures of the fragments, as shown in **Panel C**. In **Panel D**, the XICs for GCDCA and GDCA show minimal to no interference when the EAD-specific fragments are used.

To test whether this technique can be used to measure bile acids in plasma, the same 10-minute, EAD-based method used for **Figure 7** was applied to the analysis of human plasma extracts (**Figure 1).** In contrast to the results with neat standards, in the plasma matrix, the isomers GCDCA and GDCA are not baseline resolved when using a common fragment—in this case, the precursor-to-precursor MRMHr transition. However, when the data were processed using the unique EAD-based fragments, the individual bile acids were completely resolved, allowing for accurate quantitation.

In the data reported here, the ZenoTOF 8600 system was able to measure bile acids in human plasma with ~10-fold greater sensitivity than the ZenoTOF 7600 system using CID-based fragmentation. Of note, for this particular assay, a choice has to be made whether to maximize sensitivity and run the samples using the longer gradient in the negative ion mode with CID-based fragmentation, or to maximize speed and specificity by running the assay with the fast gradient using EAD-based fragmentation and sacrifice ~ 100-fold sensitivity compared to the longer assay. For the isomers in question here, GDCA, GCDCA, and GUDCA, the EAD-based method was appropriate for biological analysis. Currently, a study is underway to measure amino acid conjugates of bile acids. These molecules are measured in the positive ion mode and are amenable to a combined analysis of CID- and EAD-based analysis, using EAD only when needed to improve specificity.

Conclusions

- **The ZenoTOF 8600 system is ~ 20-fold more sensitive for the analysis of bile acids compared to the ZenoTOF 7600 system narrow XIC window possible on HRMS systems enables the MS1-based collection of data without the inclusion of chemical background interferences**
- **EAD-based fragmentation provides structural characterization of molecules and may abrogate the need for extensive chromatographic separation of isomers to ensure specificity**

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