Quantitative and qualitative analysis of bile acids by high-resolution mass spectrometry using electron-based fragmentation

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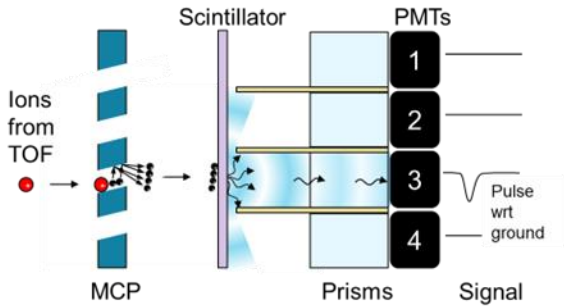
SCIEX
The power of precision

The ZenoTOF 8600 system

Optical-based detector

Optical detector with 25 psec detection rate. High-speed pulse counting to maintain resolution and mass accuracy over *5 orders LDR



Dry pumps

Higher sustainability with dry pump roughing pumps

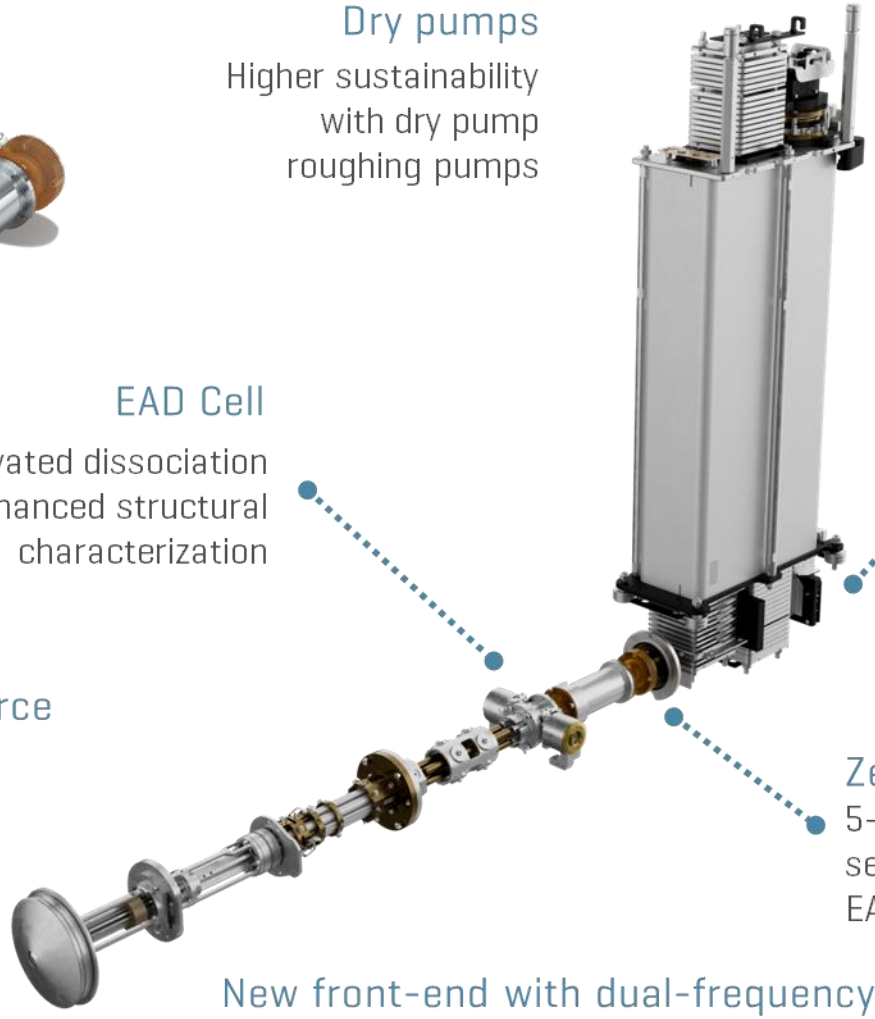
EAD Cell

Electron-activated dissociation for enhanced structural characterization

TOF & accelerator optics

Redesigned TOF apertures and horizontal steering for improved transmission and lower base pressure

OptiFlow Pro ion source



Zeno trap

5-20x gain in MS/MS sensitivity coupled with either EAD or CID fragmentation

New front-end with dual-frequency QJet & Mass Guard Technology

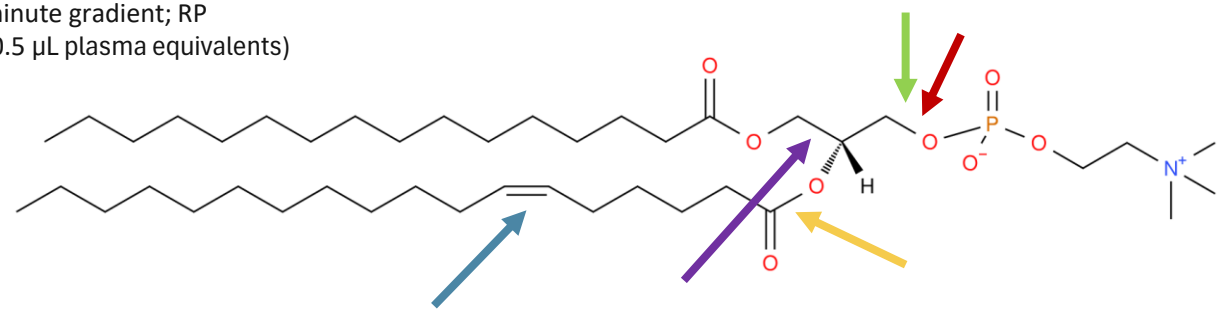
Improved ion transmission and robustness

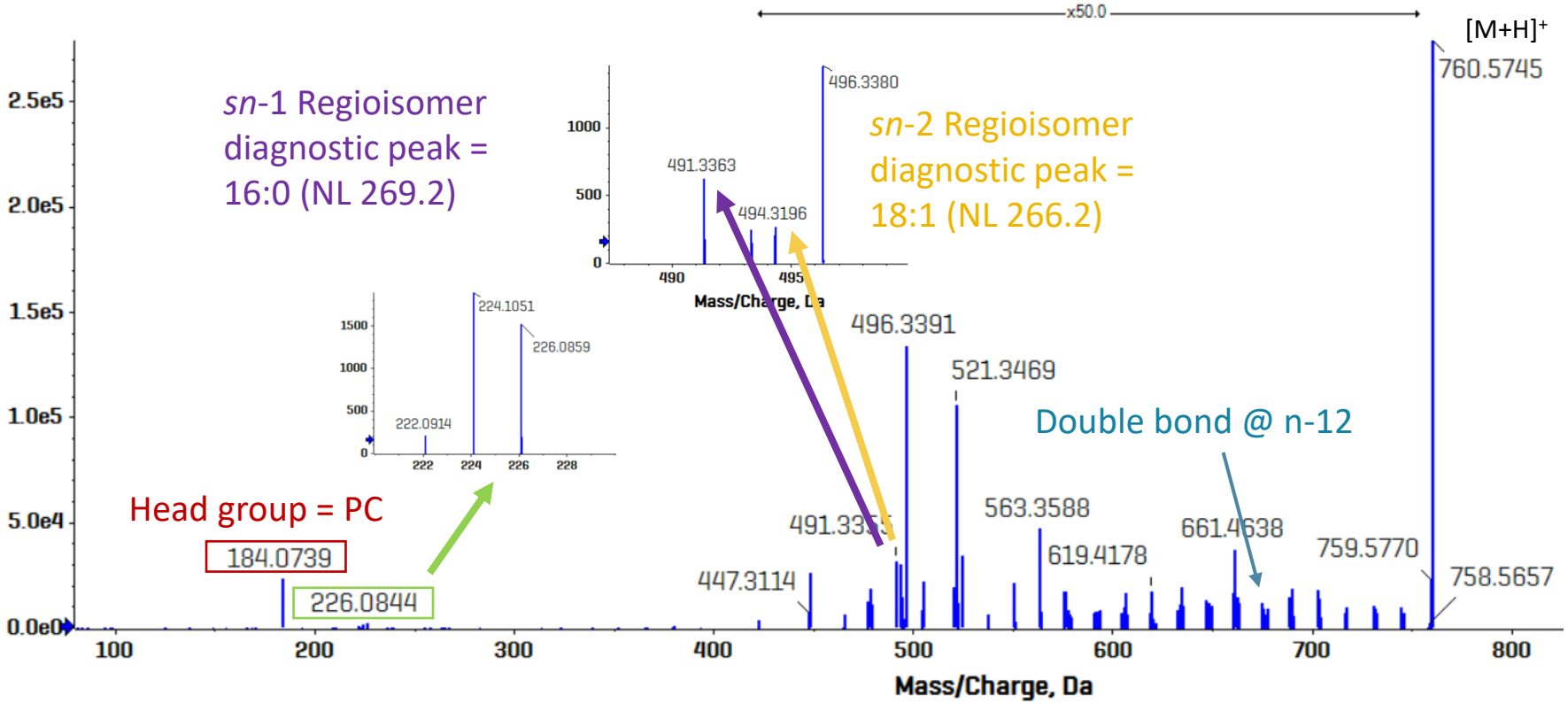
Electron-activated dissociation (EAD) as a tool for structural characterization

- CID is a thermal fragmentation technique that generates relatively few fragments
- EAD generates ~15 times the number of fragments as CID, all of which provide structural details
- Using EAD, it is possible to achieve complete structural characterization

DDA experiment on human plasma → *de novo* analysis → PC 16:0 / 18:1(n-12)

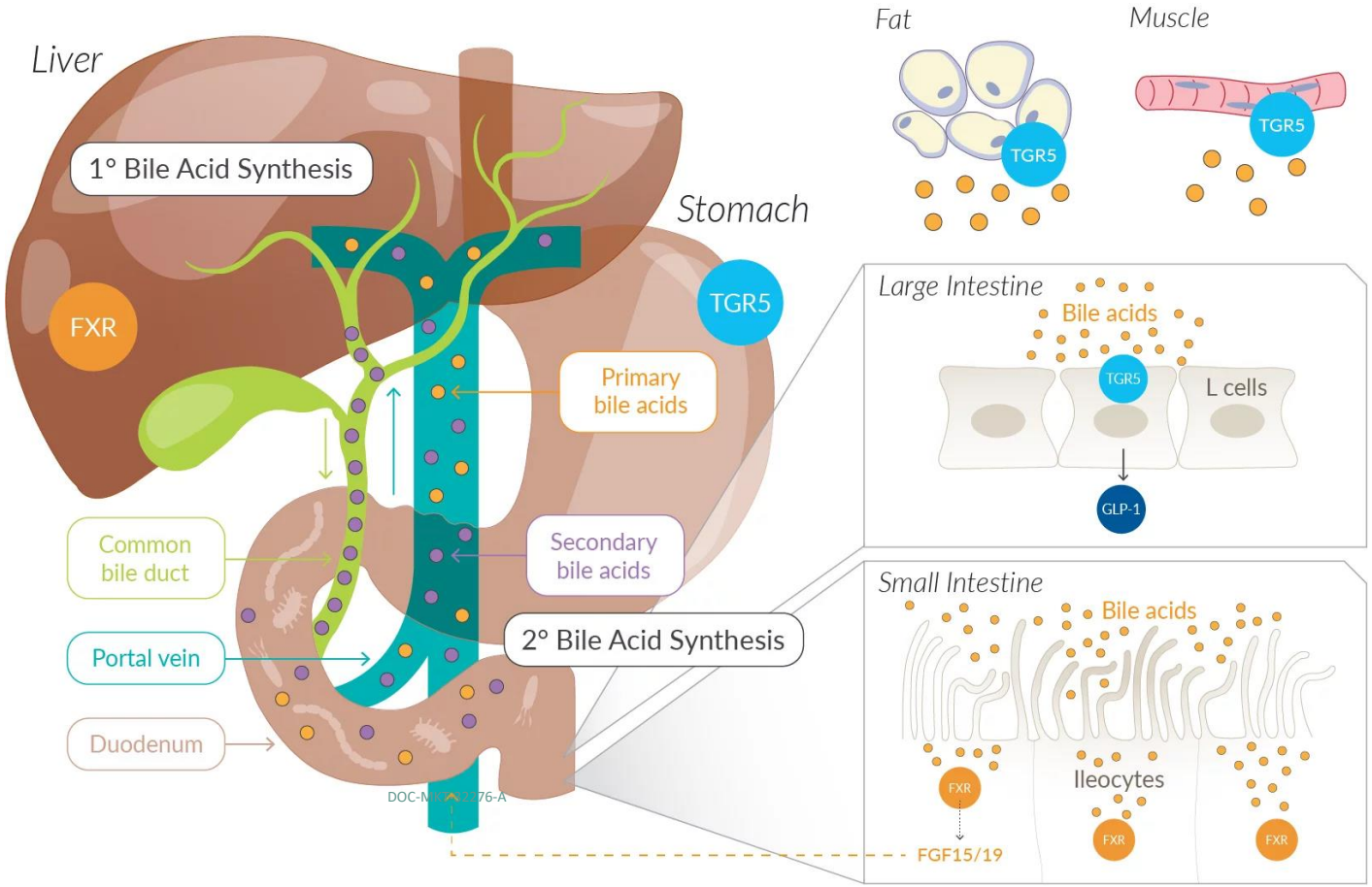
(Top-50 DDA; 17-minute gradient; RP Chromatography; 0.5 µL plasma equivalents)





Bile acids

- Primary bile acids are synthesized in the liver and pre-concentrated in the bile
- Bile is secreted into the duodenum, where bile acids play a crucial role in emulsifying and absorbing dietary fats
- The two primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA) are bioconverted into chemically distinct, bacterially produced secondary bile acids
- There is growing interest in the biomedical community to understand the pathophysiology of these secondary bile acids



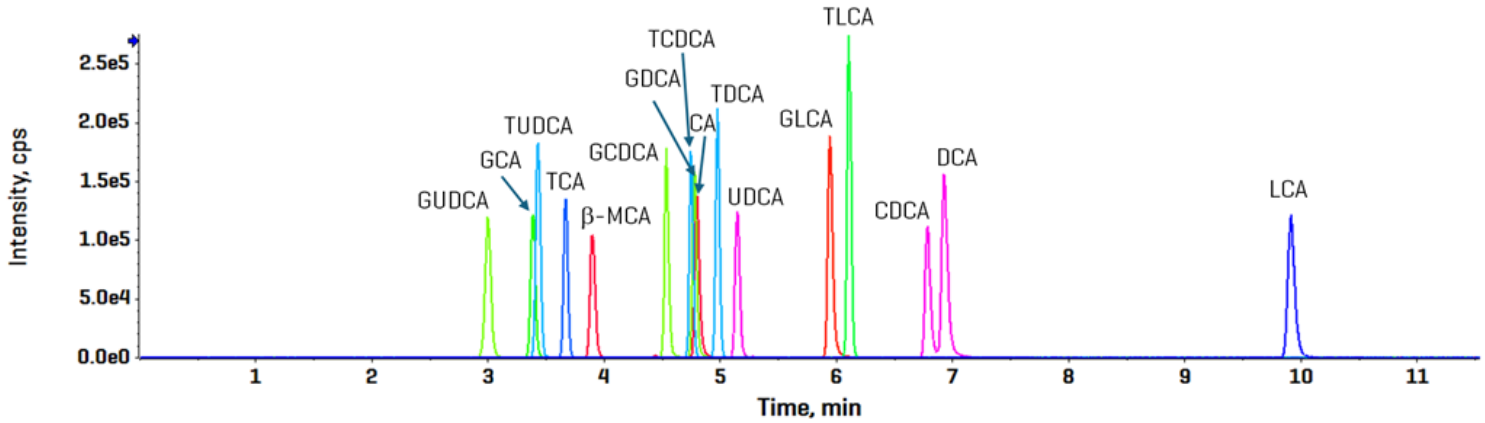
Targeted analysis of bile acids using the ZenoTOF 8600 system: experimental details

The assay needs to be sensitive and highly specific for bile acid isomers for good quantitative performance

Restek Raptor C18 column (100 x 2.1 mm; 5.0 µm particle size).

17 min gradient (CID)

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



Targeted analysis of bile acids using CID-based fragmentation in a 50 nM standard preparation

HPLC method was designed by Dr. Thomas Horvath


nature protocols PROTOCOL

Interrogation of the mammalian gut–brain axis using LC–MS/MS-based targeted metabolomics with in vitro bacterial and organoid cultures and in vivo gnotobiotic mouse models

Thomas D. Horvath, Sigmund J. Haidacher, Melinda A. Engevik, Berkley Luck, Wenly Ruan, Faith Ihekweazu, Meghna Bajaj, Kathleen M. Hoch, Numan Oezguen, Jennifer K. Spinler, James Versalovic and Anthony M. Haag

Nat Protoc. 2023;18(2):490-529

The specificity of this assay is dependent on highly developed chromatographic separation. **Can we go faster?**

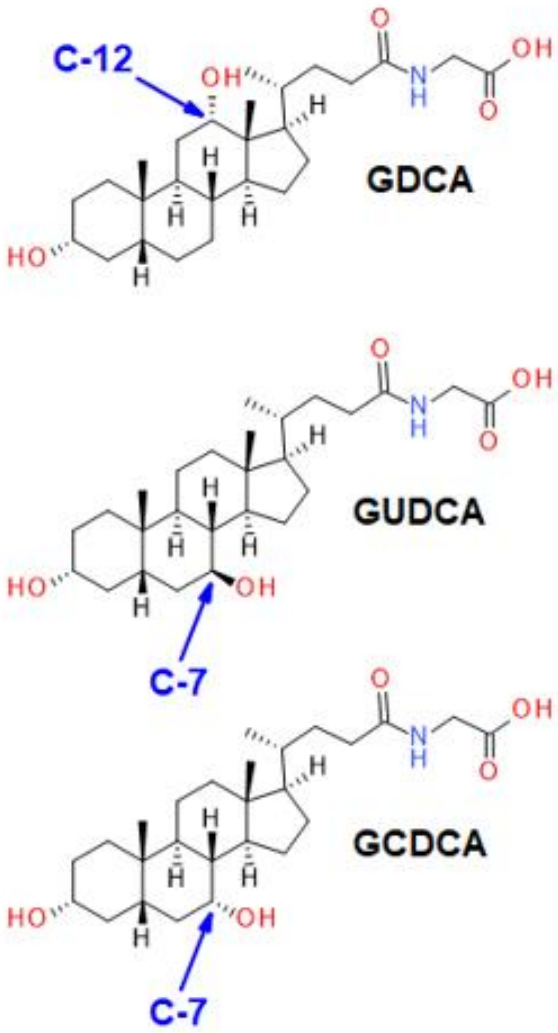
EAD has been shown to generate unique fragments among isomers, which would provide the necessary specificity without solely relying on chromatography

10 min gradient (EAD)

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

Targeted bile acid analysis: the challenge of isomers

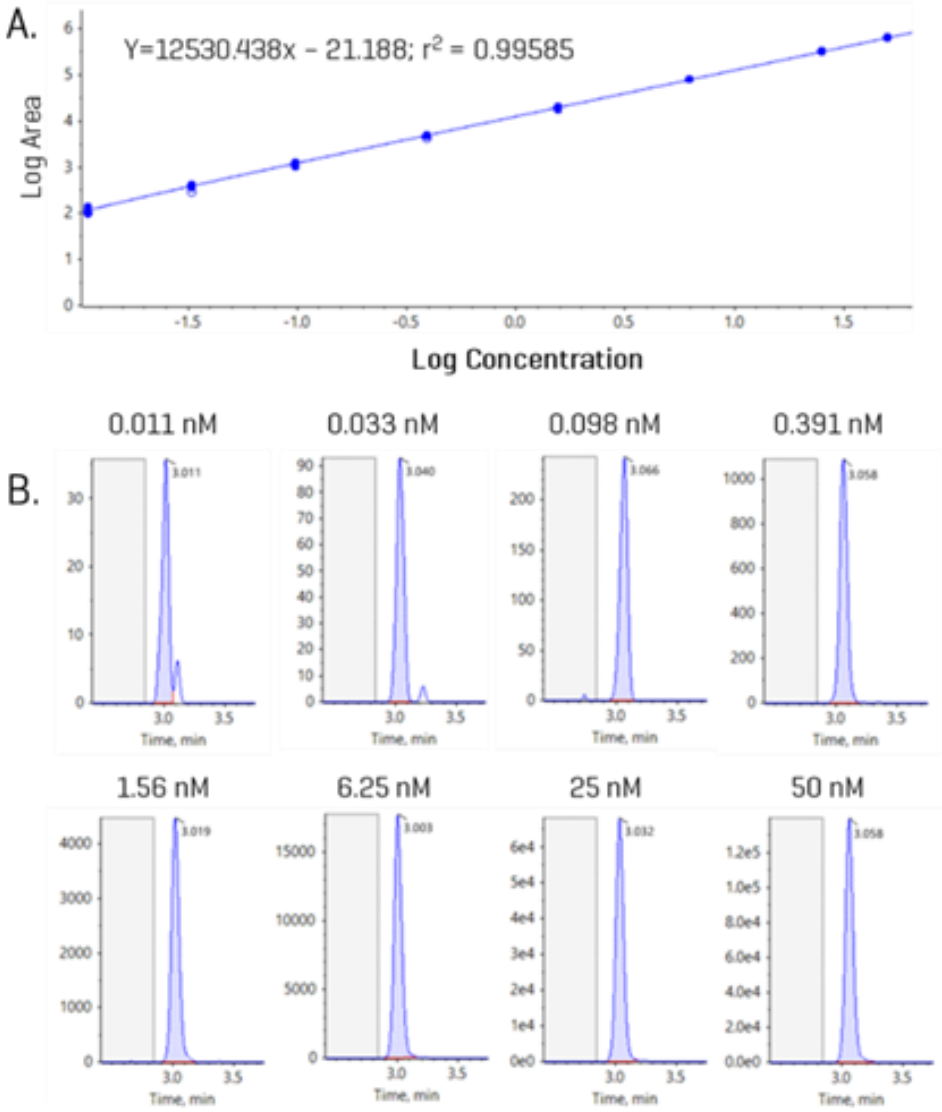
GDCA, GUDCA, and GCDCA have the same precursor masses and identical CID-based product ion spectra



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

The analysis of bile acid standards using the MRMHr scan mode (CID-based fragmentation)

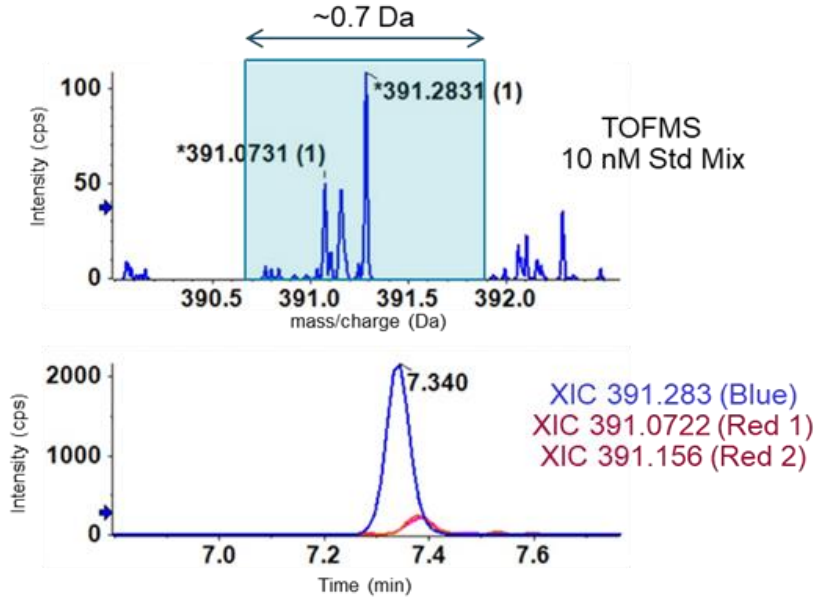
Example internal standard curve with representative peaks for GUDCA



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

Linear dynamic range > 4.5 orders of magnitude



- The high sensitivity of the ZenoTOF 8600 system is augmented by using a narrow XIC window, which reduces background interferences

Comparative quantitative performance of the ZenoTOF 7600 and 8600 systems

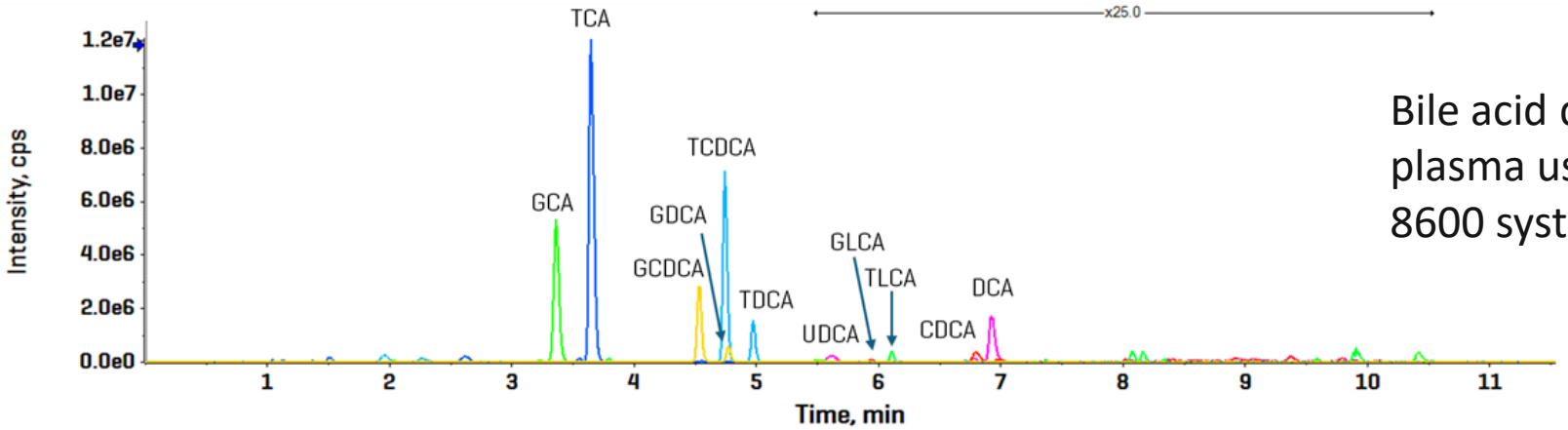
LOQ values for bile acids on the ZenoTOF 8600 system

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

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
	ZeonTOF 7600 system	8600 ZenoTOF 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

In vivo analysis of bile acids in human plasma samples (CID-based fragmentation)

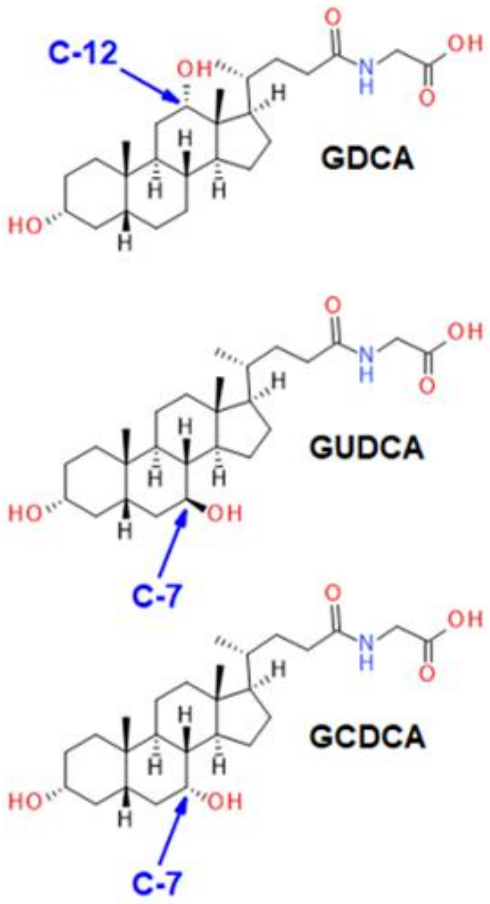


Bile acid detection and quantitation in human plasma using sMRMHr analysis on the ZenoTOF 8600 system using CID-based fragmentation

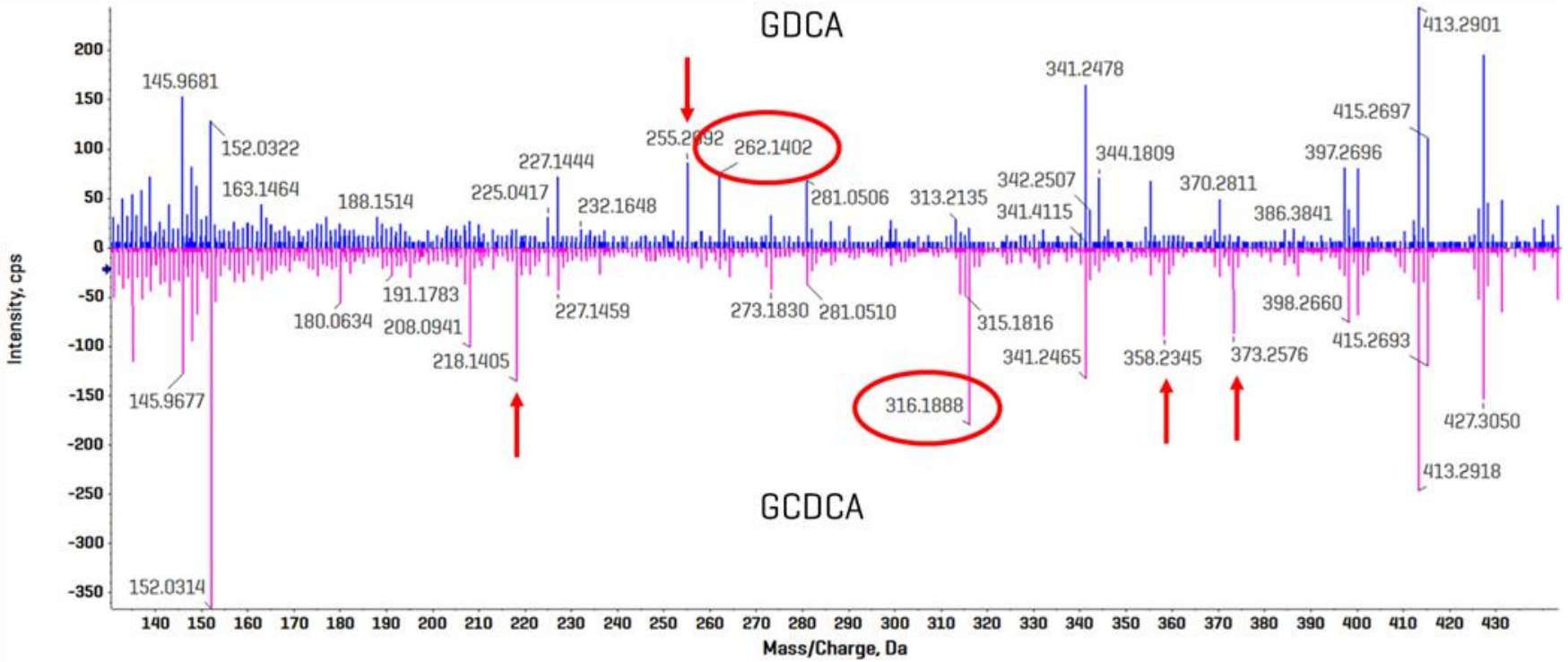
Example quantitative results from 2 different human plasma samples

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

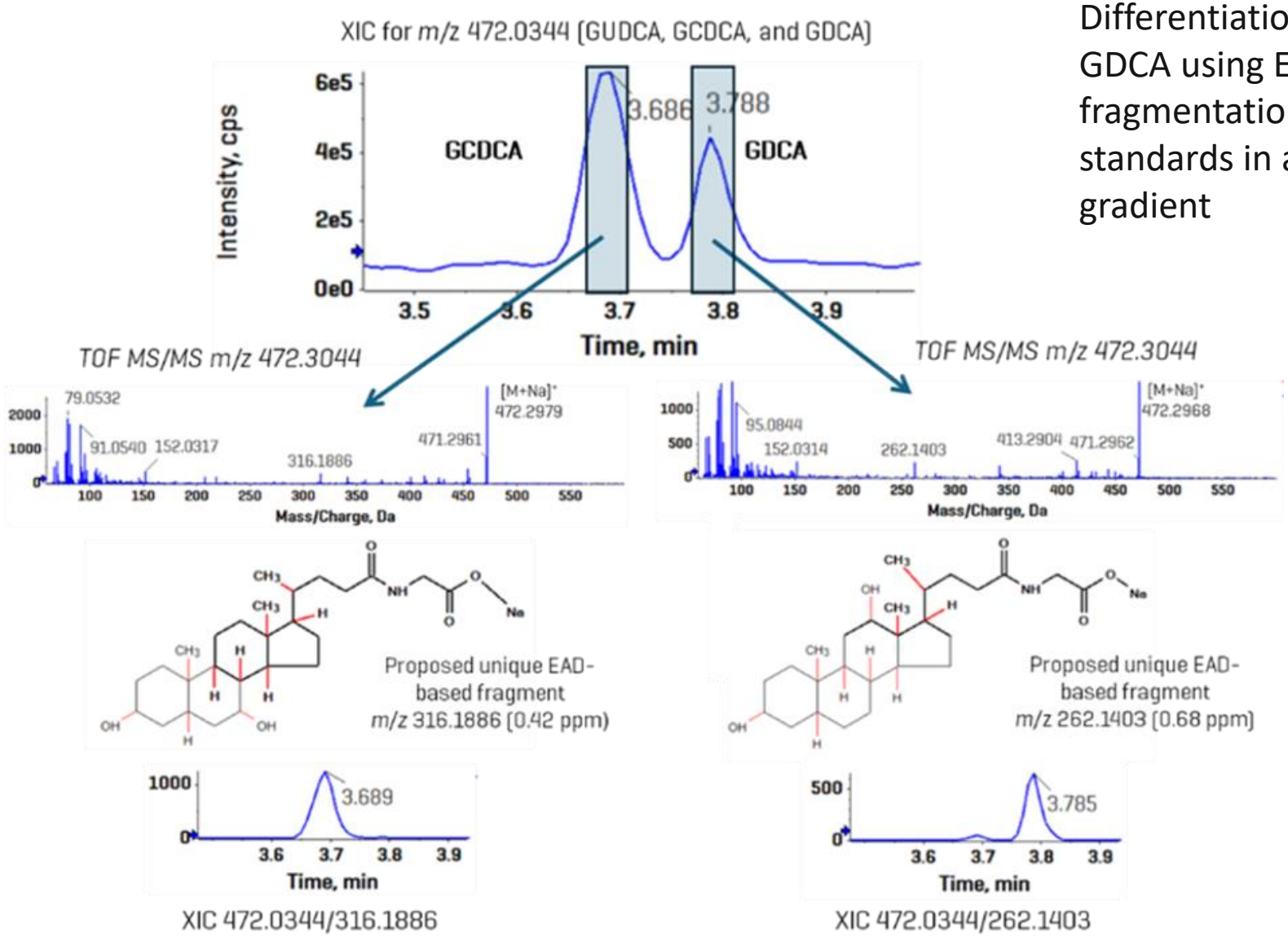
Analysis of bile acids using EAD-based fragmentation



Identification of unique, structurally diagnostic fragments among bile acid isomers to improve quantitative specificity



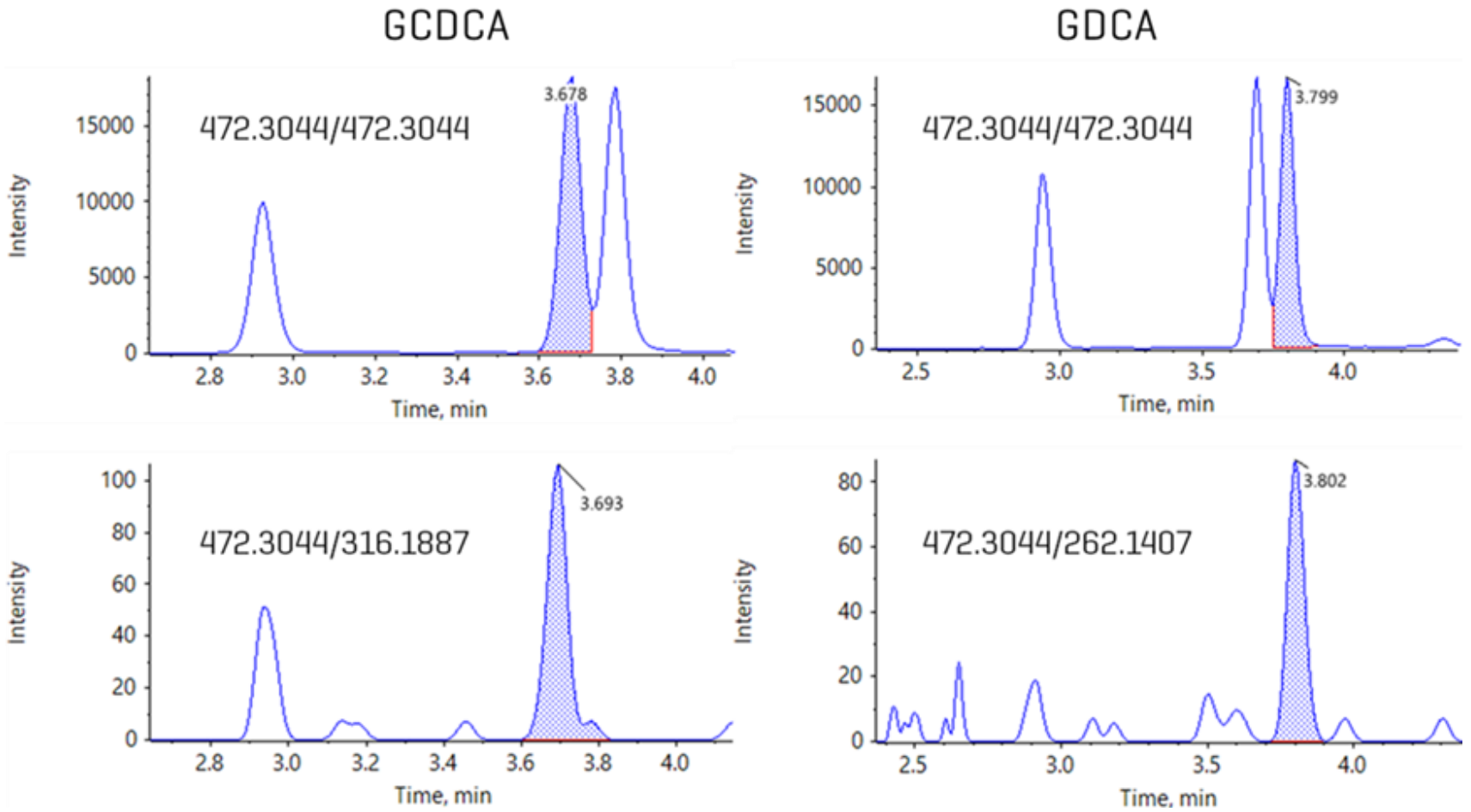
Analysis of bile acids using EAD-based fragmentation



Differentiation of GCDCA and GDCA using EAD-based fragmentation on neat standards in a 10-minute gradient

Improved specificity of bile acid analysis with faster analysis speeds

Specific detection and quantitation of GCDCA and GDCA in human plasma using EAD-based quantitation, analyzed with a 10-minute gradient



Conclusions

- **The ZenoTOF 8600 system is an ideal platform for small-molecule quantitation**
  - The sensitivity of the system is >10-fold better than the ZenoTOF 7600 system

At the TOF MS/MS level, the increased sensitivity generates more intense product ion

- spectra for CID- and EAD-based fragmentation experiments
- The improved sensitivity provides better quantitative performance in targeted MRMHr analysis; data from multiple experimental conditions suggest a 2-3 fold improvement in sensitivity compared to the SCIEX 75 00+ system
- EAD reaction times can be reduced compared to the ZenoTOF 7600 system, due to the improved sensitivity, and still provide dramatically improved qualitative analysis of biomolecules

Thank you