High resolution ion mobility timsTOF Pro for the fast separation and characterization of isomeric bile acids

Xuejun Peng[1], Lucy Woods[2], Beixi Wang[1], Surendar Tadi[2], Erica Forsberg[1].

[1]Bruker Scientific LLC, 61 Daggett Drive, San Jose, CA 95134, USA; [2]Bruker Scientific LLC, 40 Manning Road, Billerica, MA 01821, USA

Introduction

Bile acids can facilitate the digestion and absorption of lipids in the small intestines, regulate cholesterol homeostasis, and ultimately eliminate it via the fecal route. Its structural or spatial isomers can exhibit different chemical activity, potency, toxicity, and behavior in biological systems. The separation of bile acid isomers has been challenging due to their complex nature and low concentrations in biological matrices such as plasma, urine and feces. Chromatography (HPLC, GC, SFC, TLC), capillary electrophoresis and derivatization reactions are commonly used techniques for isomer separation but can be costly and time consuming. Recently, ion mobility separations of bile acid isomers was reported [1, 2] but additives were required to form adducts or applying LC separation [3]. In this work, a trapped ion mobility (TIMS) timsTOF Pro workflow was established for the fast separation of bile acid isomers without using additives or LC separation. Ions of bile acid isomers are separated in an inert gas phase and opposing electric field based on the difference of shape and sizes subjected to a potential gradient.

Methods

Bile acid isomers of lithocholic acid (LCA), isolithocholic acid (iso-LCA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), and isodeoxycholic acid (iso-DCA) (Sigma, Avanti) were dissolved individually in methanol at 50 µg/mL as stock solutions, which were further diluted with methanol at 1.0 µg/mL individually or separately as the working solution; data was acquired for 30 seconds with direct infusion or flow injection by Elute UHPLC - timsTOF Pro 2 (Bruker) in ESI negative mode with TIMS and PASEF enabled; data was processed in DataAnalysis 5.3 . An established 4D-Metabolomics method (m/z 20-1300 Da; ion mobility 1/K0 0.45 – 1.45 V.s/cm2) was applied and optimized to achieve the highest ion mobility resolution by adjusting the TIMS parameters of duty cycle, ramp time, and ion mobility range etc. Both mass (TOF) and CCS (TIMS) calibration were performed prior to sample analysis.

Results and Discussion

The chemical structure of bile acid DCA and LCA isomers are listed in Figure 1.

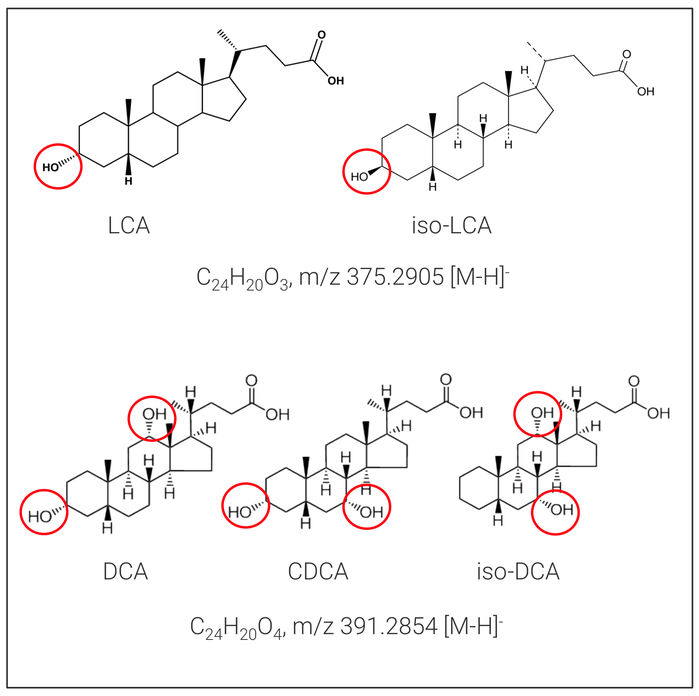

Figure 1. Chemical structure of bile acid isomers

Bile acids	Abbrev.	Molecular Formula	$[M-H]^-$	Mobility 1/K0	CCS (Å^2)	IM Resolution	CCS Reference value	ΔCCS %
Deoxycholic acid	DCA	$C_{24}H_{40}O_4$	391.2854	0.969	200.9	166.7	202.1[a]	0.59
Chenodeoxycholic acid	CDCA	$C_{24}H_{40}O_4$	391.2854	0.9947	206.2	176.7	201.5[b]	-2.33
Isodeoxycholic acid	iso-DCA	$C_{24}H_{40}O_4$	391.2854	0.9608	199.2	171.6	199.2[a]	0.00
Lithocholic acid	LCA	$C_{24}H_{40}O_3$	375.2905	0.9952	206.6	171.8	201.4[a]	-2.58
Isolithocholic acid	iso-LCA	$C_{24}H_{40}O_3$	375.2905	0.9922	203.9	175.3	202.3[c]	-0.79

(a) Unified CCS Compendium (https://mcleanresearchgroup.shinyapps.io/CCS-Compendium/); (b) PucChem (https://pubchem.ncbi.nlm.nih.gov/); (c) MetCCS Database (http://www.metabolomics-shanghai.org/MetCCS/search/)

Table 1. Ion mobility resolution and CCS for bile acid isomers

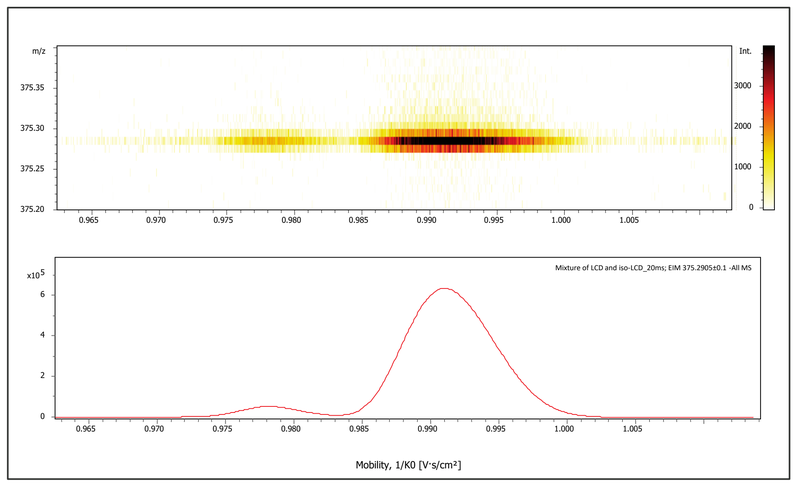

Figure 2. Heatmap of LCA and iso-LCA mixture

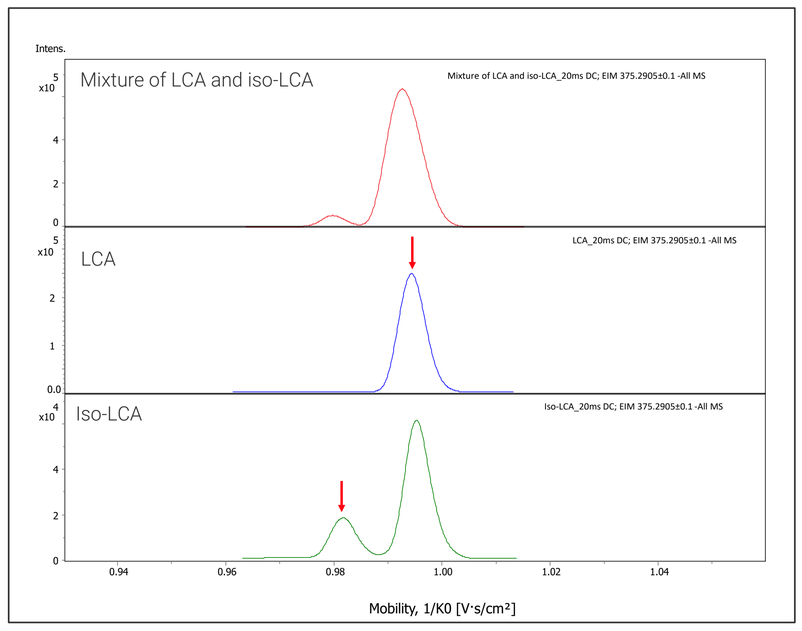

Figure 3. EIM of LCA and iso-LCA

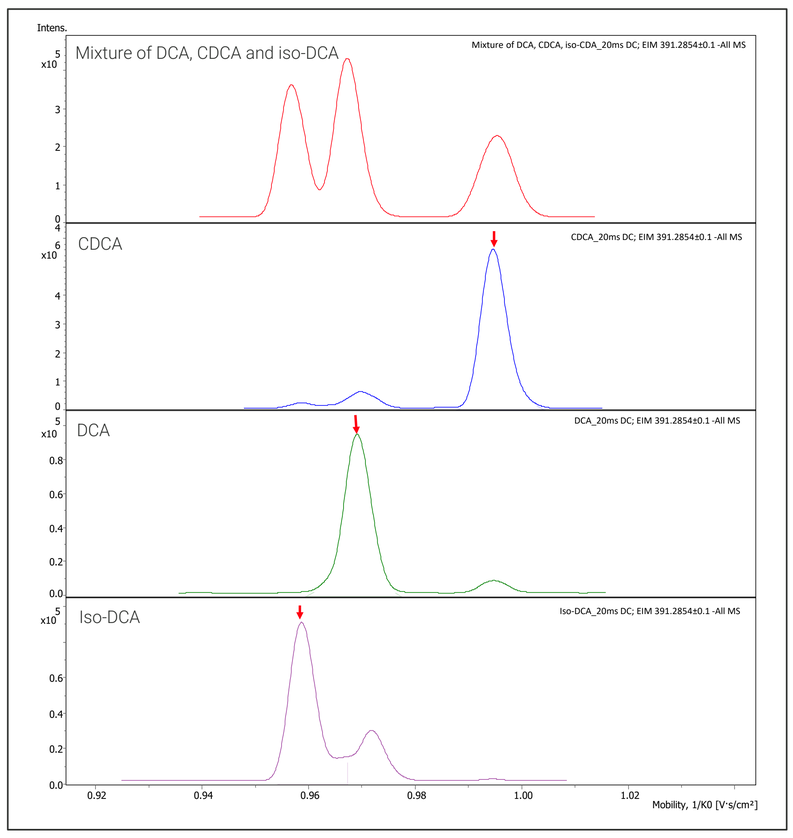

Figure 4. EIM of DCA, CDCA and iso-DCA

The ion mobility separation of bile acid isomers and its heat maps were displayed in figures 2-5, and the ion mobility separation resolution and CCS value for each bile acid isomer were listed in table 1.

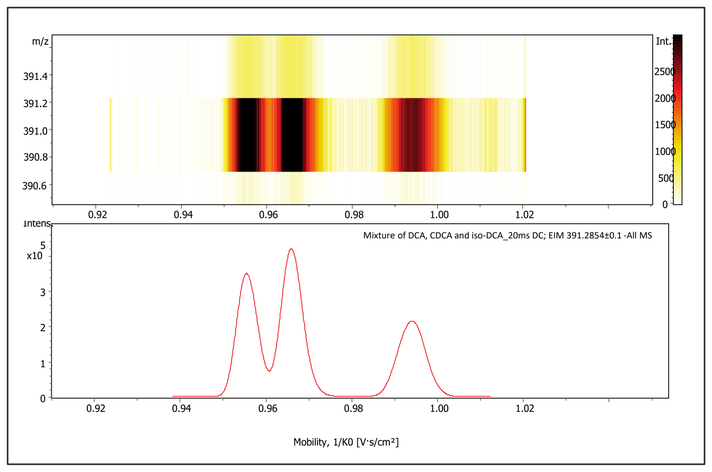

Figure 5. Heatmap of DCA, CDCA and iso-DCA mixture

References

1) Richard D. Smith et al., *Anal. Chem*., 2018, 90(18): 11086-11091
2) John A. McLean et al., *J Am Soc Mass Spectrom*, 2020, 31 (8): 1625-1631
3) https://sciex.com/tech-notes/life-science-research/lipidomics/separation-of-bile-acid-isomers-with-differential-mobility-spect

Conclusion

- Ion mobility separation of bile acid isomers were achieved by direct infusion or flow injection timsTOF Pro
- A high throughput analytical method for profiling and separation of isomers

Ion Mobility/timsTOF Pro