

Effects of Increased Fructose Consumption and Inadequate Copper Intake on the Pathogenesis of Nonalcoholic Fatty Liver Disease (NAFLD): A Feces, Plasma and Liver Metabolomics Study

David E. Alonso¹, Biyun Shi², Ming Song², Xinmin Yin², Xiaoli Wei², Michelle Page¹, Joe Binkley¹, Craig McClain², and Xiang Zhang²
¹ LECO Corporation, St. Joseph, MI USA | ²Departments of Chemistry and Medicine, University of Louisville, KY USA

Introduction

Introduction

Inflammation, oxidative stress, dyslipidemia, insulin resistance, and obesity are key clinical risk factors for the progression of nonalcoholic fatty liver disease (NAFLD).¹ Interactions between diet, liver, and immune system play an important role in this disease.² Increased fructose consumption and inadequate copper intake are two critical factors that may contribute to metabolic syndrome and lead to the development of diseases such as NAFLD.³ For example, increased consumption of high fructose corn syrup has been associated with liver fibrosis severity in subjects with NAFLD.⁴ Metabolomics is a viable method for identifying compounds associated with NAFLD. In this investigation, high quality data facilitate the identification of key metabolites differentiating normal versus diseased species.

Objectives

- To further investigate the relationship between copper deficiency and fructose-induced fatty liver disease.
- Implement the use of enhanced, comprehensive chromatography and high resolution time-of-flight mass spectrometry for separation and confident identification of metabolites in samples

Technology

Sample Introduction

Data Acquisition & Processing

LECO Pegasus[®] GC-HRT & HRT 4D with ChromaTOF-HRT[®] Software



Instrument Attribute | **Value**

Mass Accuracy	<1 ppm
Mass Range	10-1500 m/z
Resolving Power	up to 50,000
Data Acquisition Speed	Up to 200 sps
Ionization	EI, PCI

Experimental

Samples

84 Samples, six groups:

Group	Copper Level	High Fructose Diet
1	Adequate	Yes
2	Low	Yes
3	Supplemental	Yes
4	Adequate	no
5	Low	no
6	Supplemental	no

Samples were extracted with methanol and water and derivatized using a two step process:

- Methoximation (30 μL of MEOX, 60°C, 1 hr)
- Silylation (20 μL MTBSTFA, 60°C, 1 hr)

Instrument Parameters

Gas Chromatograph	Agilent 7890 with Dual Stage Quad Jet Modulator and MPS2 Autosampler
Injection	1 μL, Split 20:1, 280°C (1μL Splitless for CI)
Carrier Gas	He @ 1.0 ml/min, Constant Flow
Column 1	Rxi-5 ms, 60 m x 0.25 mm i.d. x 0.25 μm (Restek, Bellefonte, PA, USA)
Column 2	Rxi-17 sil ms, 0.60 m x 0.25 mm x 0.25 μm coating (Restek, Bellefonte, PA, USA)
Temperature Program	0.5 min at 60°C, ramped 5°C/min to 270°C, held 6 min Secondary oven maintained +10°C relative to primary oven
Thermal Modulation (GCxGC)	4 s with temperature maintained +15°C relative to 2nd oven
Mass Spectrometer	LECO Pegasus [®] HRT 4D
Ion Source Temperature	250 °C (EI), 200 °C (CI)
Acquisition Mode	High Resolution, R = 25,000 (FWHM)
Ionization Mode	EI and or CI (Reagent Gas: 5% NH ₃ in CH ₄)
Mass Range (m/z)	30-510 (EI), 60-1500 (CI)
Acquisition Rate	10 spectra/s (200 spectra/s GCxGC)

Processing & Statistical Methods

- Experimental data was processed using ChromaTOF brand software (Peak Selection, Compound Identification). Compounds were rapidly and confidently identified using high quality data and effective software tools.
- Statistical processing was performed using MetPP Software (Alignment, Metabolite Quantification).
- 38 compounds were identified as significant in determining the relationship between copper and fructose intake. These included acids (lactic acid, 3-hydroxybutyric acid, 2-aminobutyric acid), amino acids (aspartic acid, cysteine, proline, glutamic acid, ornithine), diacids (succinic acid, malic acid, fumaric acid), and fatty acids (myristic, pentadecanoic acid, palmitic acid).

GC-HRT

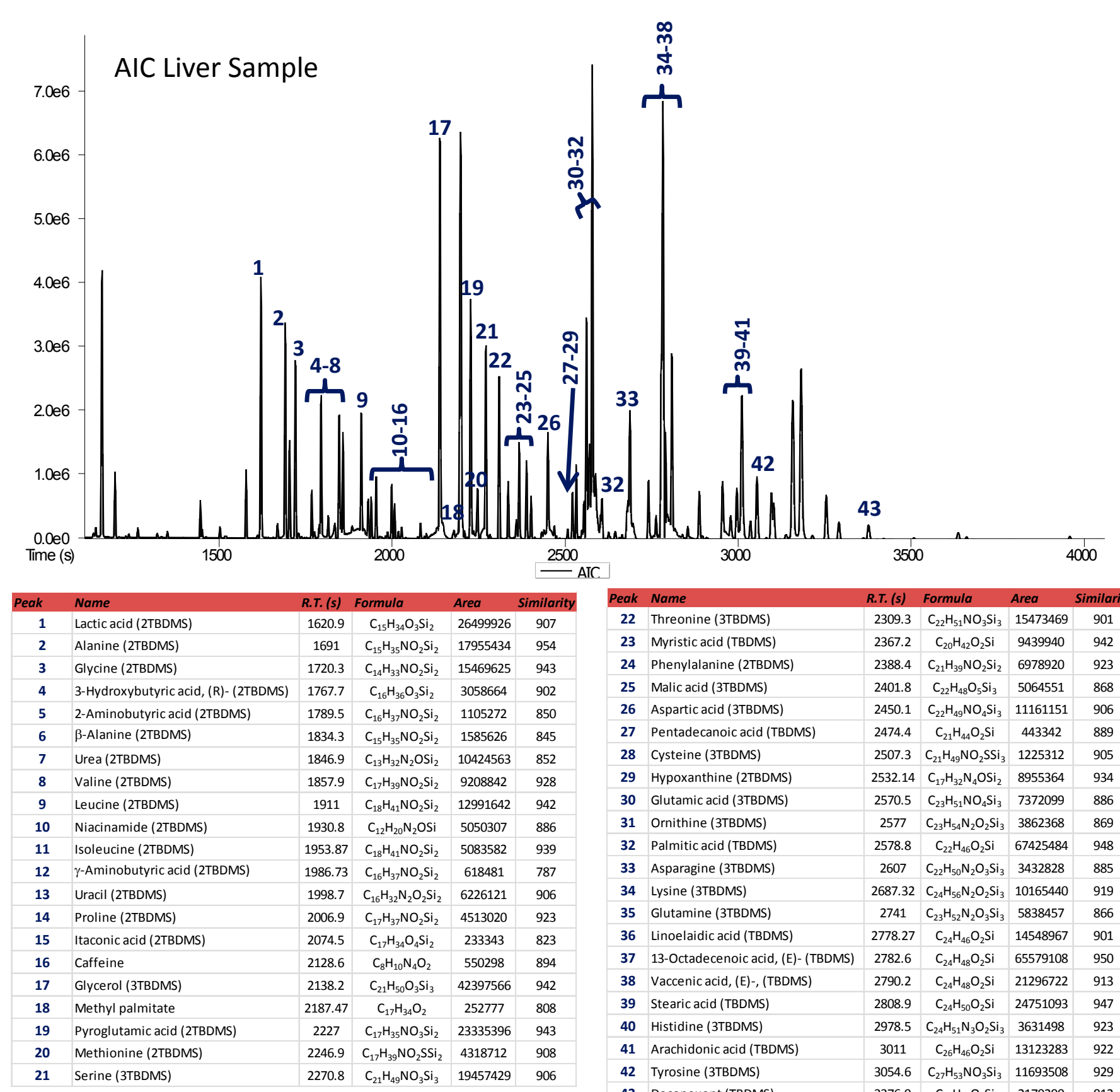


Figure 1. AIC and Table of representative compounds in liver tissue. Metabolites were automatically identified through ChromaTOF-HRT processing.

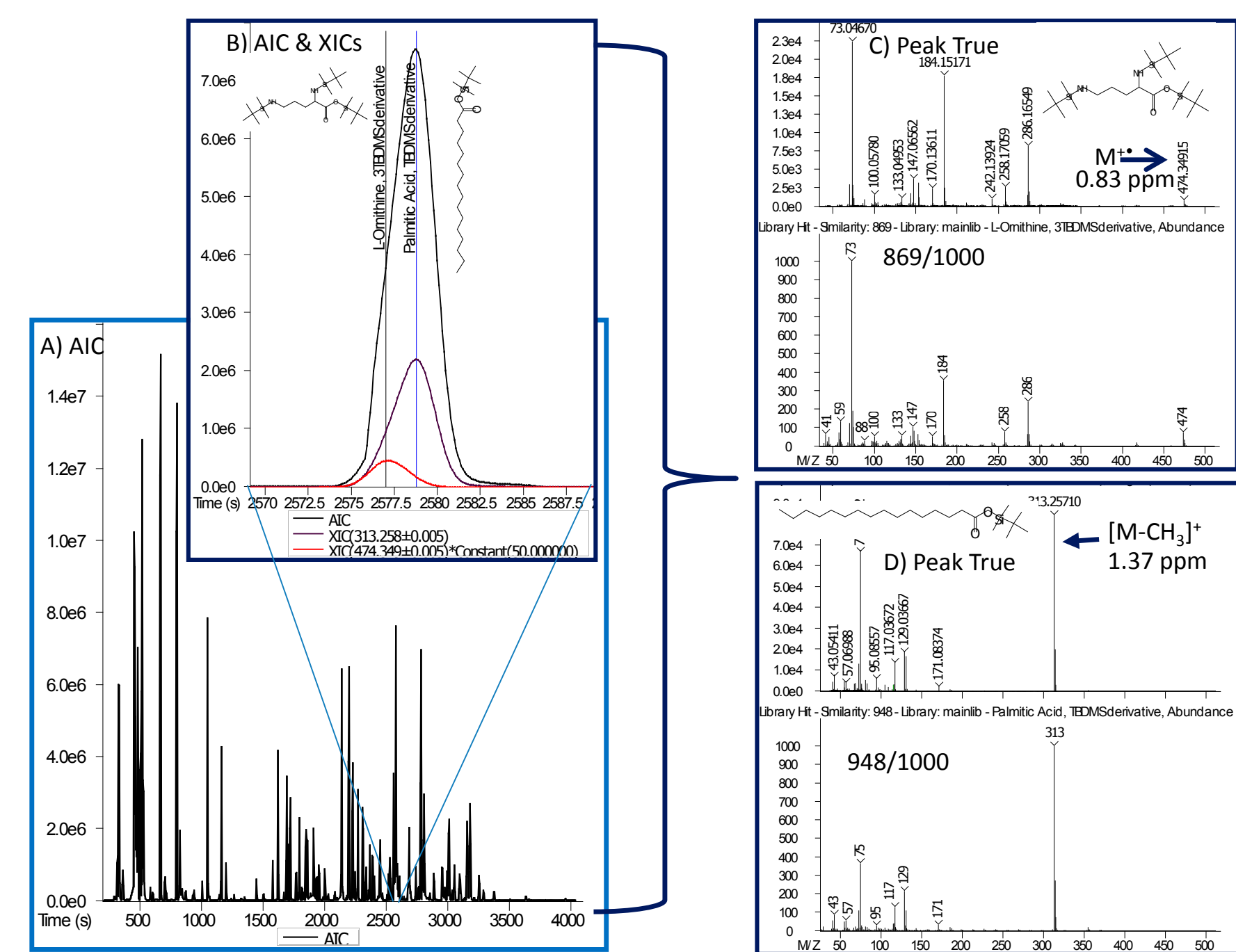


Figure 2. AIC and XIC expansion of liver tissue demonstrating benefits of High Resolution Deconvolution. Peak true and library mass spectral data for ornithine & palmitic acid.

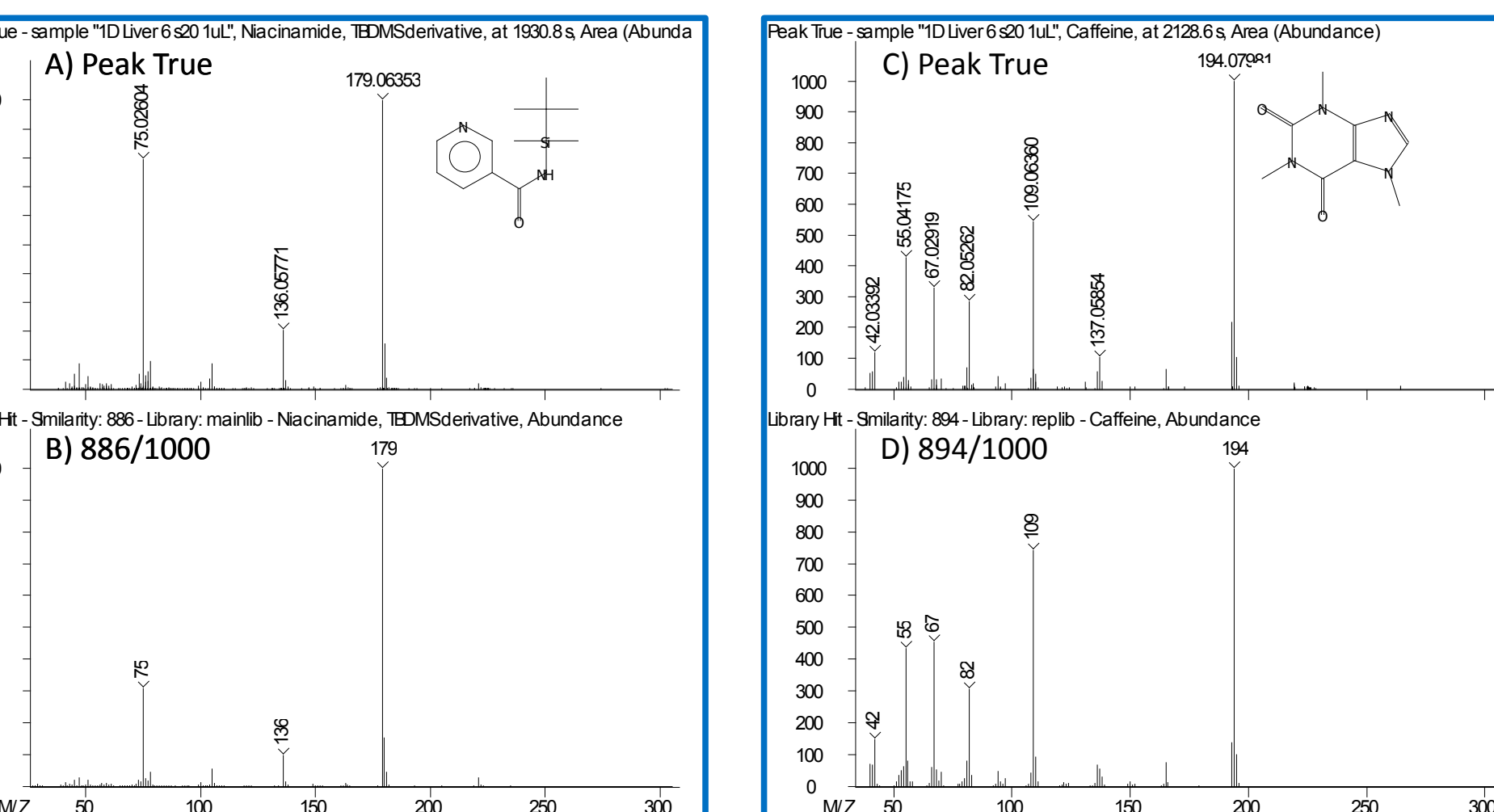


Figure 3. Peak true and library mass spectral data for niacinamide and caffeine in liver tissue.

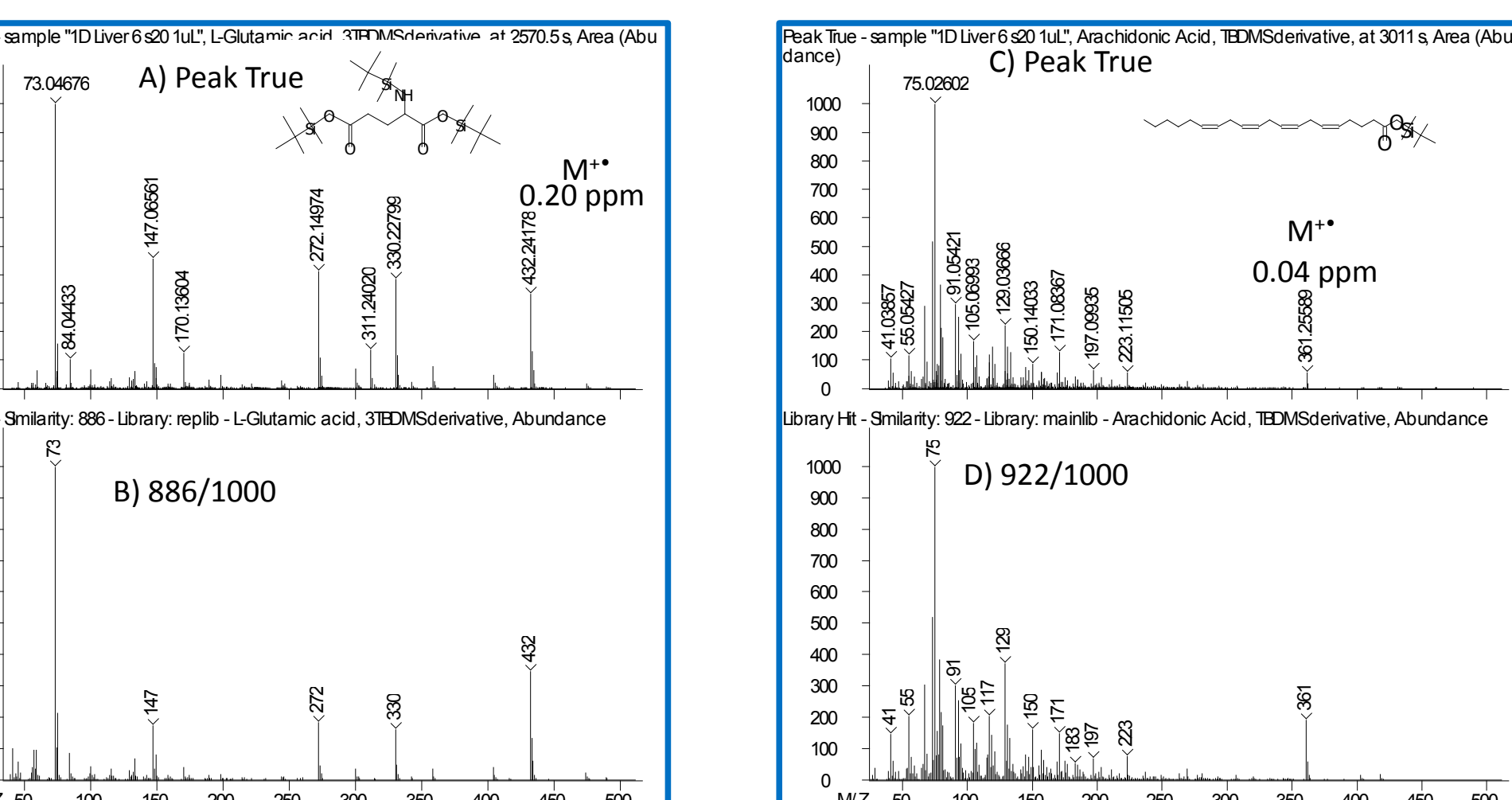


Figure 4. Peak true and library mass spectral data for glutamic acid and arachidonic acid in liver tissue. High resolution accurate mass ions (HRAM) and mass accuracy values for the molecular ions.

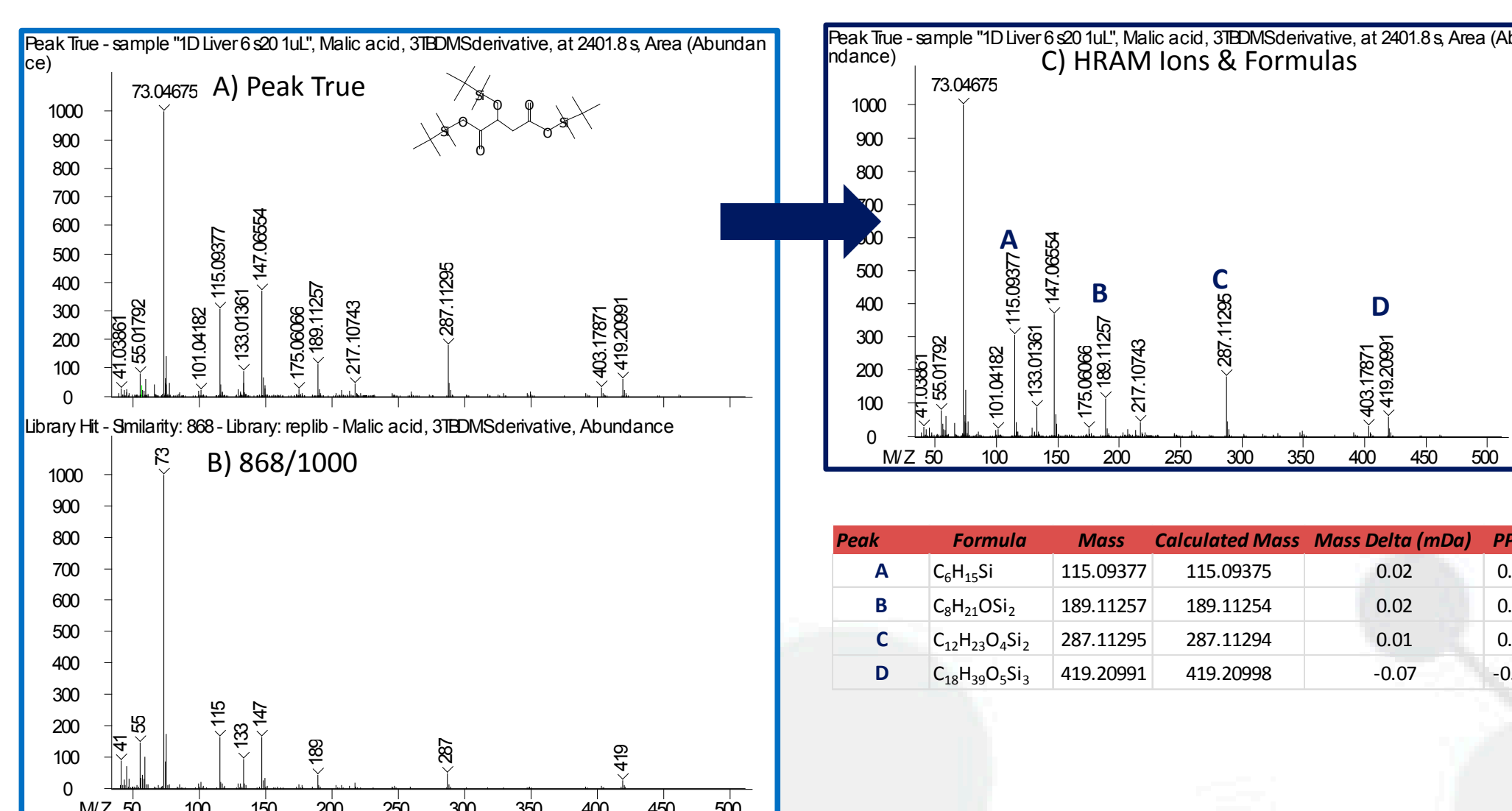


Figure 5. Peak true and library mass spectral data for malic acid in liver tissue. HRAM fragment ions, formulas, and mass accuracy values.

Complementary EI and CI-HRT Data

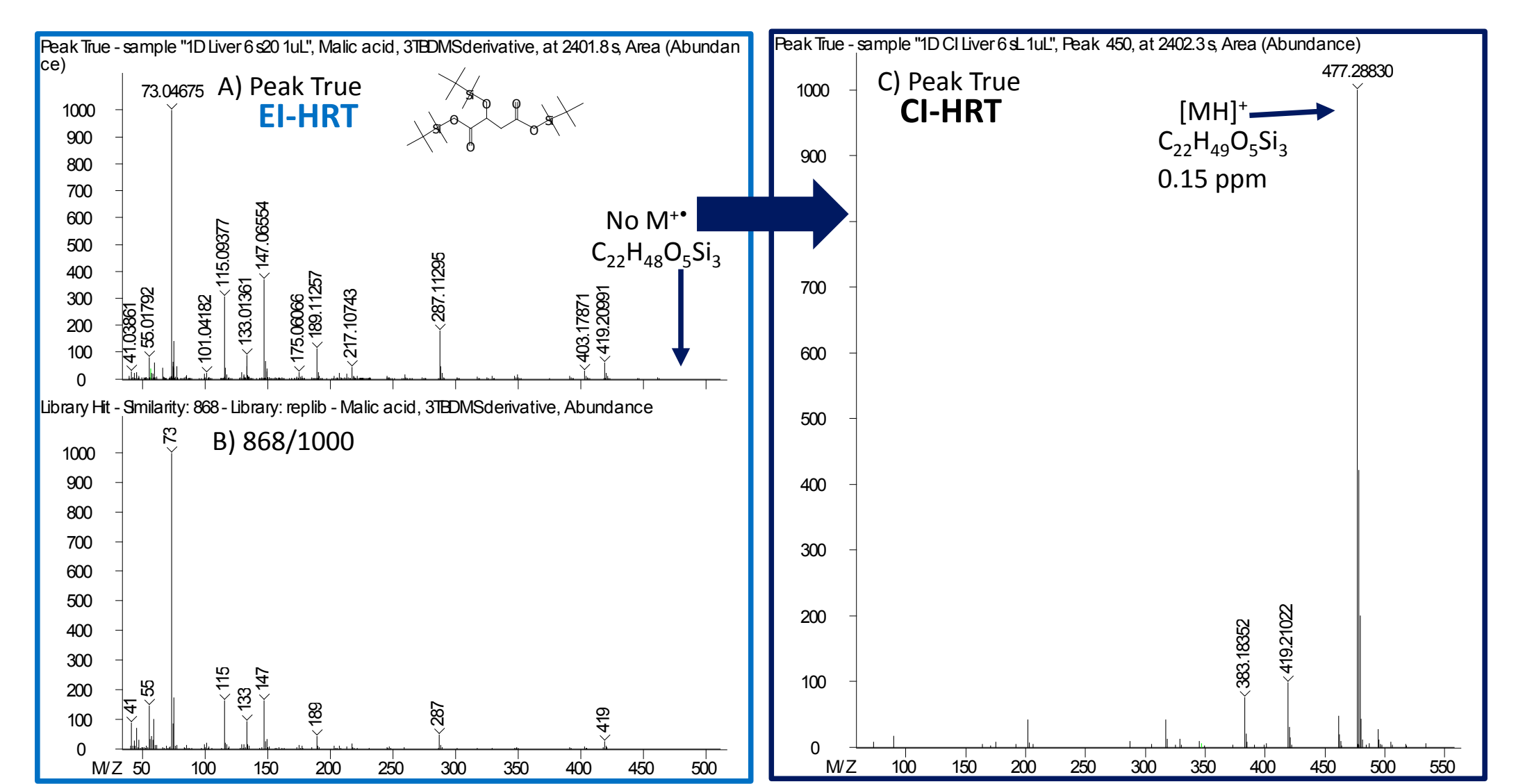


Figure 7. Peak True EI-HRT, Library, and CI-HRT Data for Malic Acid.

GCxGC-HRT

HRT 4D: GCxGC-HRTofMS

- Enhanced Chromatographic and Mass Spectral Resolution
- Group Clustering – Structured Chromatograms
- Improved Characterization of Compounds

Figure 8. GCxGC-HRT 4D Instrument

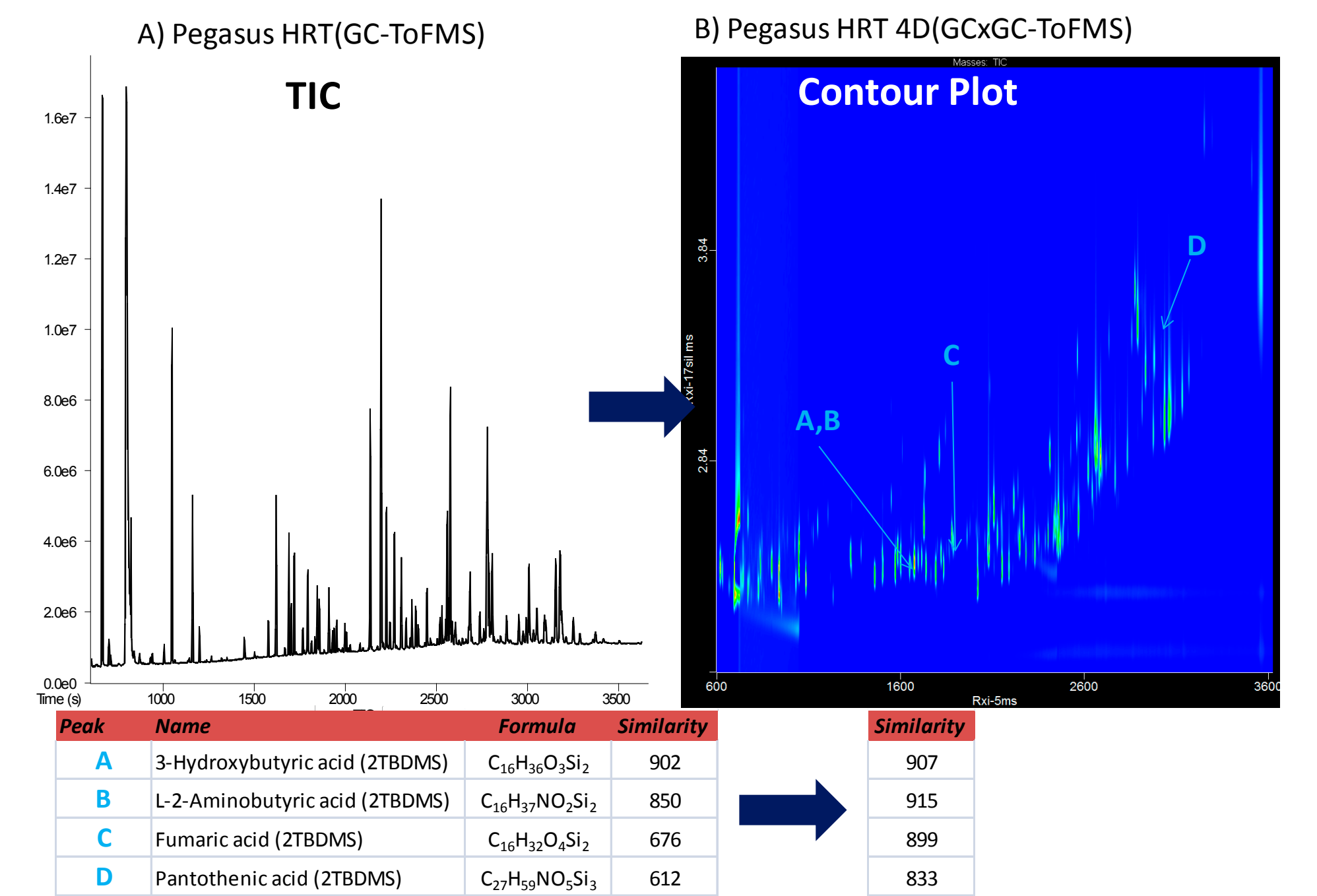


Figure 9. A) GC-HRT and B) GCxGC-HRT Data. Table illustrating the benefits of combining enhanced chromatographic and mass spectral resolution for confident identification of metabolites differentiating groups (e.g., fumaric acid).

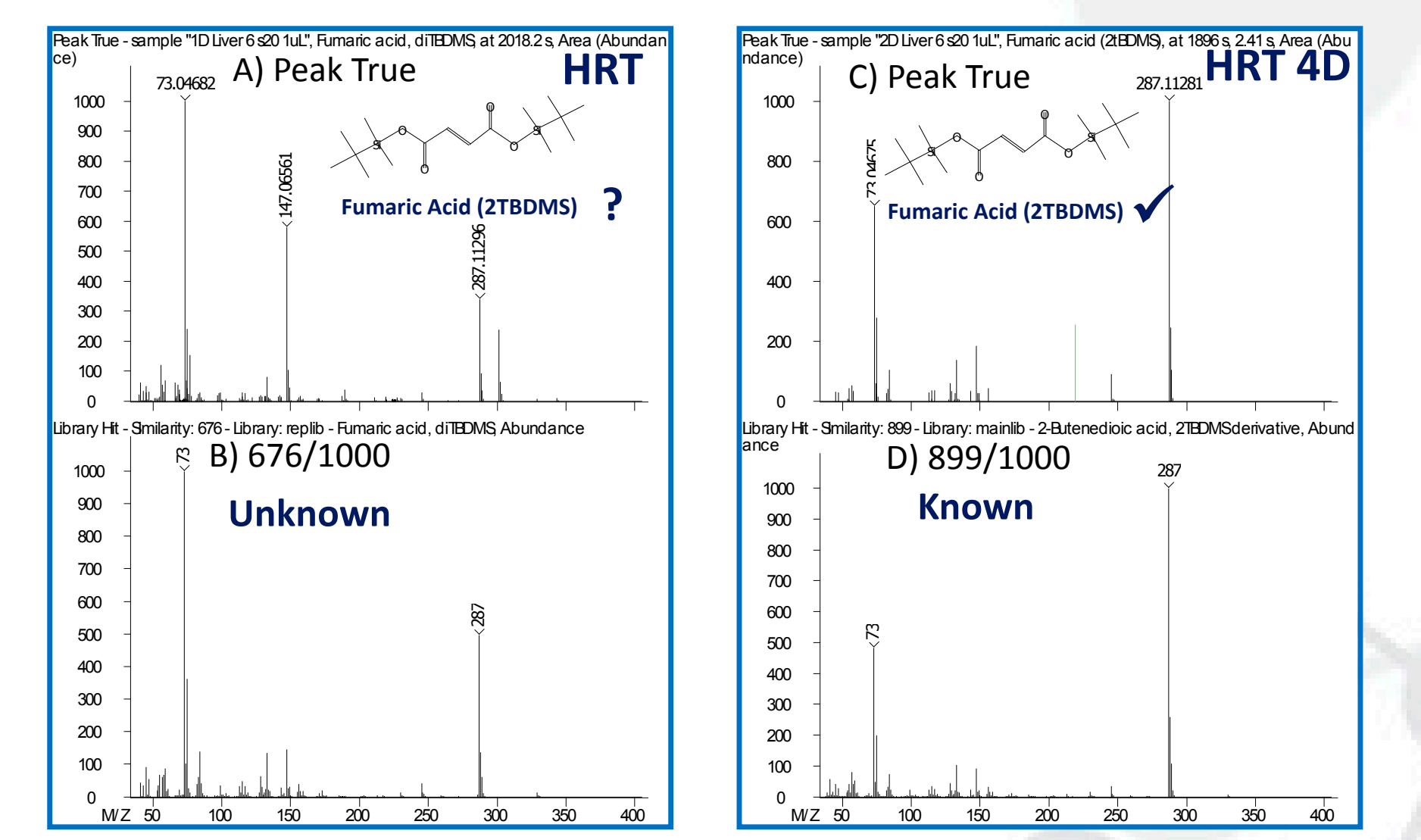


Figure 10. HRT and HRT 4D Mass Spectral Data for Fumaric Acid (Unknown → Known)

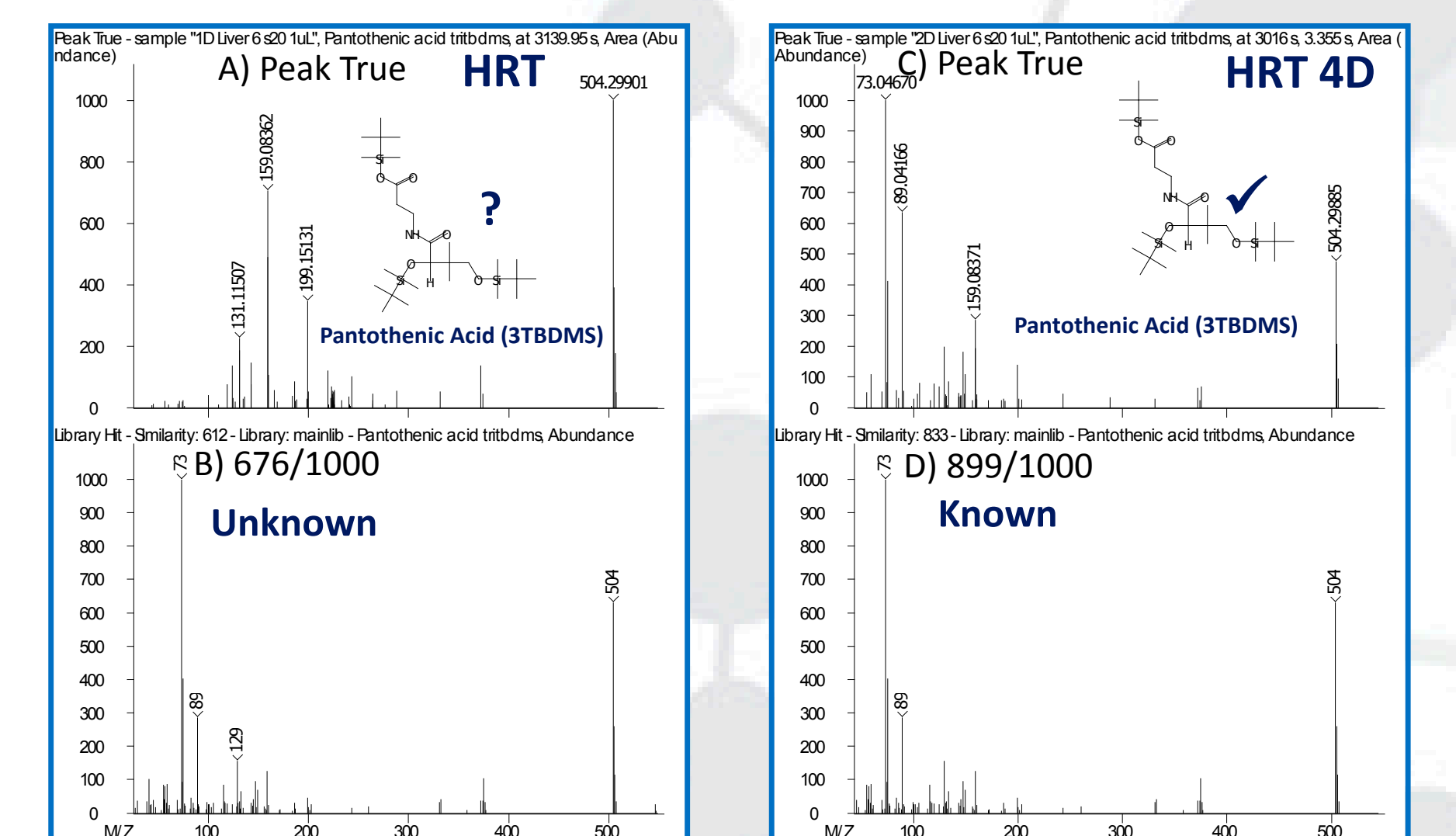


Figure 11. HRT and HRT 4D Mass Spectral Data for Pantothenic Acid (Unknown → Known)

Conclusion

- 38 metabolites detected with significant abundance alteration between the study groups were easily and automatically identified using high quality data and superior processing tools
- Enhanced two-dimensional chromatographic resolution, spectral similarity searches of large, well-established databases, and formula determinations using high resolution accurate mass ions increased confidence in metabolite characterization.

References

- Y-S, Lai, W-C Chen, T-C Kuo, C-T. ho, C-H. Kuo, F.J. Tseng, K-H. Lu, S-H. Lin, S. Panyod and L-Y Sheen, *J. Agric. Food Chem.*, **2015**, 63, 7873-7884.
- X. Shi, X. Wei, X. Yin, Y. Wang, M. Zhang, C. Zhao, H. Zhao, C.J. McClain, W. Feng and X. Zhang, *J. Proteome Res.*, **2015**, 14(2), 1174-1182.
- X. Wei, M. Song, X. Yin, D.A. Schuschke, I. Koo, C.J. McClain and X. Zhang, *J. Proteome Res.*, **2015**, 14(9), 4050-405.
- M.F. Abdelmalek, A. Suzuk, C. Guy, A. Unalp-Arida, R. Colvin, R.J. Johnson and A.M. Diehl, *Hepatology*, **2010**, 51(6), 1961-1971.