

Blood Serum Metabolomics Using Comprehensive Two-Dimensional Gas Chromatography High Resolution Time-of-Flight Mass Spectrometry

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Introduction

- Traumatic brain injury (TBI) is a major cause of neurological damage, disability, and death
- Mild, moderate, or severe (Glasgow Coma Scale)
- Metabolites are a viable measure of TBI:
 - Can pass through the blood brain barrier
 - Provide a quick measure of biological activity
 - Can be used to measure long-term effects of brain injuries (e.g., Sports)
- Moderate (M) and Severe (S) TBI Patient Samples:
 - Branched chain amino acids (Down regulated)
 - Sugar derivatives, short-chain carboxylic acids (Up regulated)
- Problem: Unidentified metabolites**
- Task => Characterize unknowns in pooled TBI serum samples:**
 - M1,M2,M3 and S1,S2,S3**

Workflow

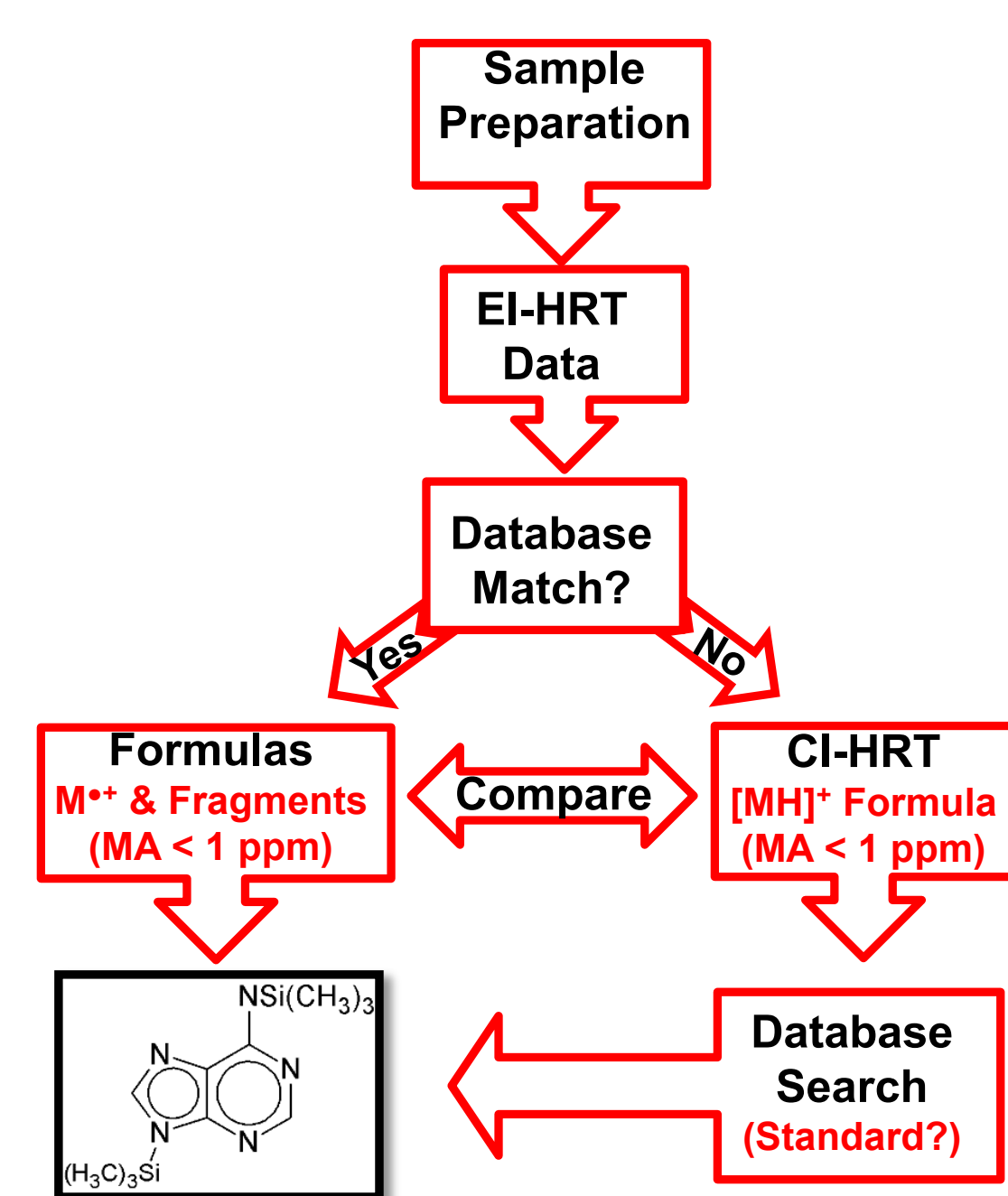


Figure 1: Metabolomics Workflow

Instrumentation

- Robust and reproducible chromatography
- EI and CI data (up to 200 sps; resolution up to 50K)
- 1D and 2D High Resolution Deconvolution® (HRD®)
- Production of high quality, accurate mass data:
 - Database searches (NIST, Wiley, Fiehn, etc.)
 - Formula searches (Fragments, molecular ions, adducts)

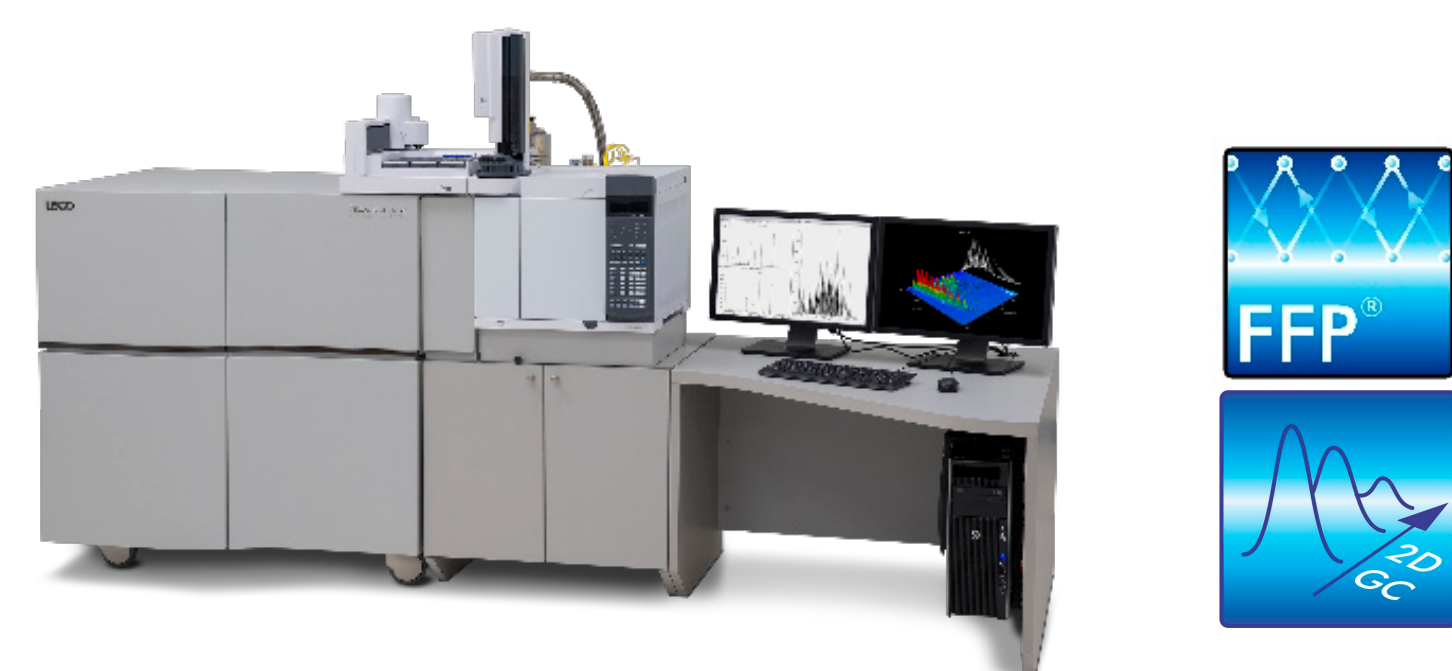


Figure 2: Pegasus® GC-HRT 4D

Methods

- Sample Preparation
 - Extraction: MeOH/H₂O
 - Derivatization:
 - 25 µL of MEOX (20 mg/mL in Pyridine)
 - Heat at 45°C for 1 hr
 - 50 µL of MSTFA
 - Heat at 45°C for 1 hr

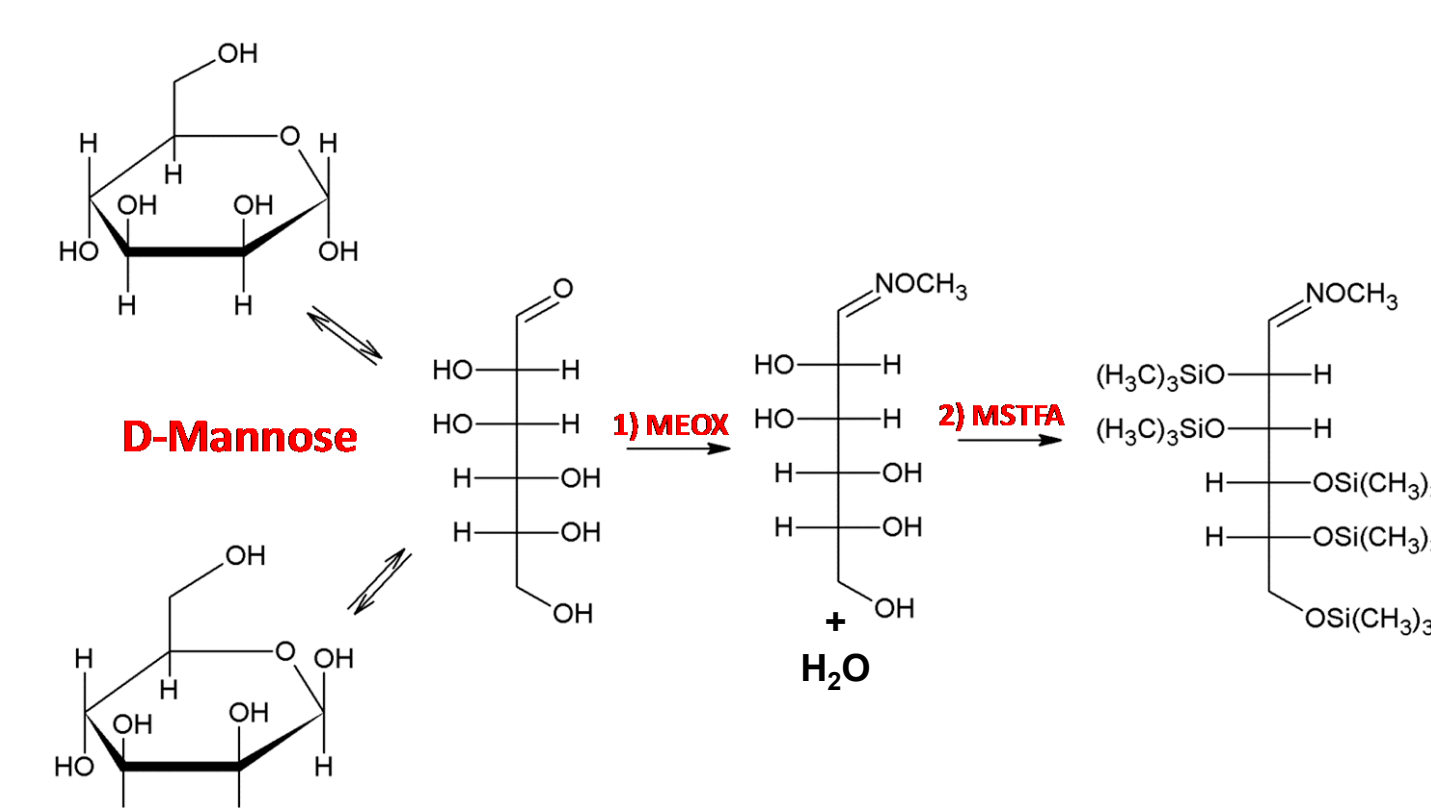


Figure 3: Derivatization increases coverage

2) Instrument Parameters

Table 1. GC, GCxGC, and TOFMS Parameters

Gas Chromatograph	Agilent 7890, Dual Stage Quad Jet Modulator, MPS2 Autosampler
Injection	0.5µL, Split 10:1, 50 to 250°C @ 12°C/s; (1µL Splitless for CI)
Carrier Gas	He @ 1.0 mL/min, Constant Flow
Column 1	Rxi-5 Sil MS, 30 m x 0.25 mm i.d. x 0.25 µm (Restek, Bellefonte, PA, USA)
Column 2	Rxi-17Sil MS, 0.60 m x 0.25 mm x 0.25 µm coating (Restek, Bellefonte, PA, USA)
Temperature Program	2 min at 70°C, ramped 10°C/min to 320°C, held 6 min Secondary oven maintained +10°C relative to primary oven
Thermal Modulation (GCxGC)	3 s with temperature maintained +15°C relative to secondary 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 CI (Reagent Gas: 5% NH ₃ in CH ₄)
Mass Range (m/z)	30-510 (EI); 60-1500 (CI)
Acquisition Rate	6 spectra/s (200 spectra/s GCxGC)

3) Data Processing

- Comprehensive (mass cal., peak find, database search)
- Target analyte finding
 - Large datasets
 - Trace analysis
 - Quantitative analysis

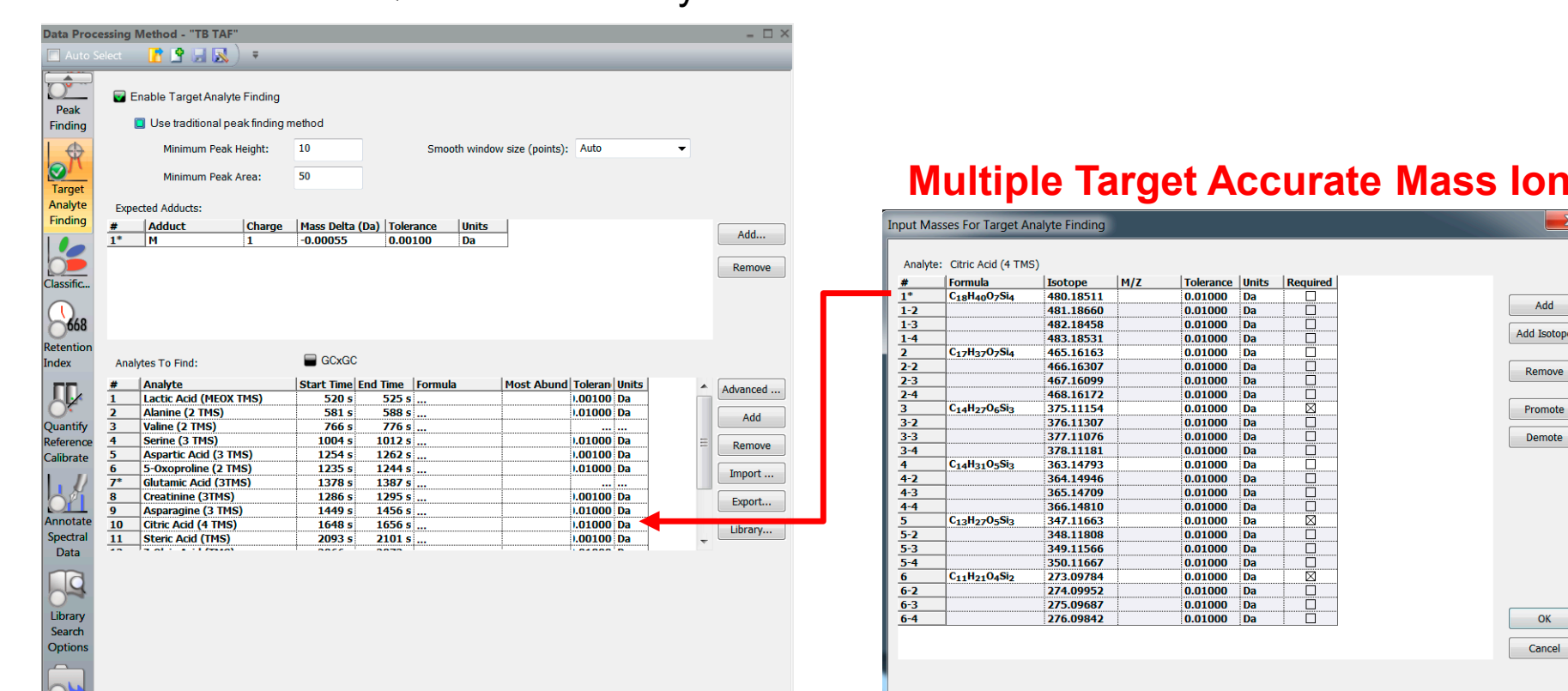


Figure 4: ChromaTOF-HRT® Target Analyte Finding

GC-HRT Results

1) Peak Find (Comprehensive) and Target Analyte Finding

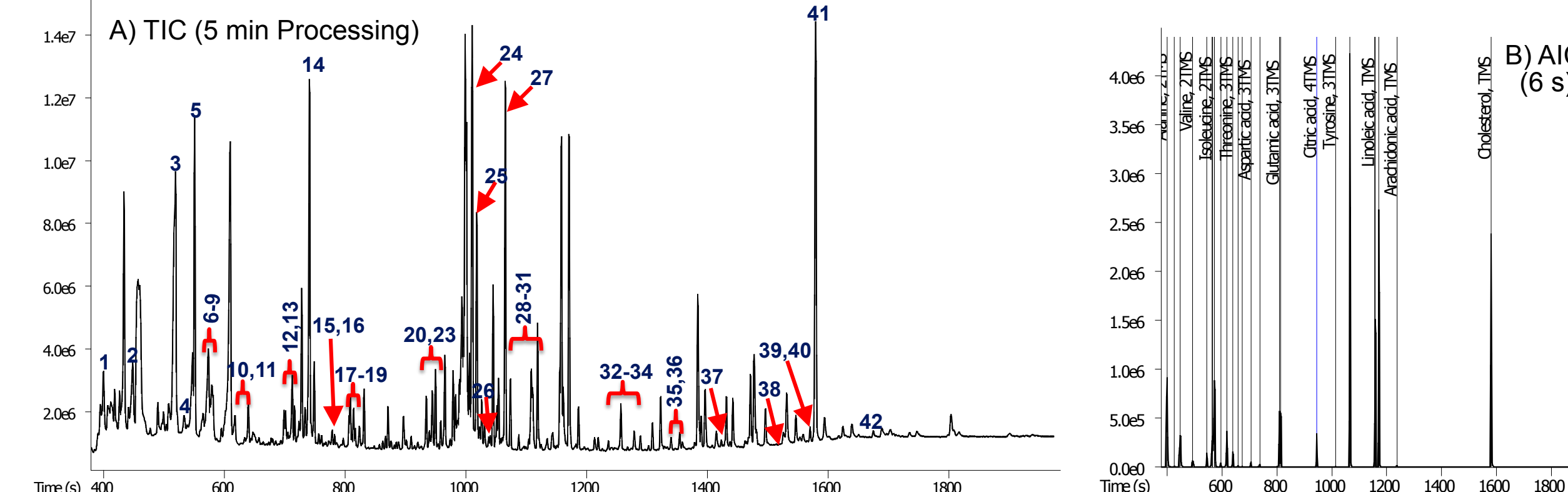


Figure 5: A) TIC sample M2, B) TAF AIC, Processing Times

Table 2. Representative compounds in M2

Peak	Name	R.T. (s)	Formula	Area	Similarity
1	L-Alanine (2TMS)	400.2	C ₆ H ₁₃ N ₂ O ₅ Si	1732787	857
2	(1S,3S)-Hydroxybutyric Acid (2TMS)	448.3	C ₆ H ₁₃ O ₅ Si	12219337	917
3	Urea (2TMS)	519.8	C ₂ H ₆ N ₂ O ₂ Si	7525045	936
4	L-Leucine (2TMS)	545.3	C ₁₀ H ₁₉ N ₂ O ₅ Si	1330591	863
5	Phosphoric Acid (2TMS)	553.2	C ₃ H ₈ O ₇ Si	3000776	905
6	Mannitol (2TMS)	585.8	C ₆ H ₁₄ O ₆ Si	89221	856
7	L-Isoleucine (2TMS)	563.3	C ₁₀ H ₁₉ N ₂ O ₅ Si	1262891	851
8	L-Proline (2TMS)	565.5	C ₆ H ₁₃ N ₂ O ₅ Si	2518767	865
9	Glycine (2TMS)	573.2	C ₂ H ₅ N ₂ O ₅ Si	1838266	840
10	Serine (2TMS)	618.2	C ₆ H ₁₃ N ₂ O ₅ Si	2664477	907
11	L-Threonine (2TMS)	640.2	C ₆ H ₁₃ N ₂ O ₅ Si	3312787	935
12	Asparaginyl Aspartic Acid (2TMS)	703.4	C ₁₀ H ₁₉ N ₄ O ₈ Si	158448	740
13	Aspic Acid (2TMS)	718.9	C ₆ H ₁₃ O ₅ Si	2954623	926
14	L-S-Deserine (2TMS)	743.5	C ₁₀ H ₁₉ N ₂ O ₅ Si	15380627	919
15	2-Oxoglutaric Acid (MEOX, 2TMS)	779.2	C ₈ H ₁₅ O ₇ Si	1468261	853
16	Propionic Acid (2TMS)	797.3	C ₄ H ₉ O ₃ Si	807051	815
17	Riboflavin (2TMS)	808.4	C ₁₇ H ₂₁ N ₄ O ₆ Si	153787	759
18	L-Glutamic Acid (2TMS)	809.1	C ₆ H ₁₃ N ₂ O ₅ Si	3348392	907
19	Phenylalanine (2TMS)	814.4	C ₉ H ₁₃ N ₂ O ₅ Si	2487612	840
20	Citric Acid (2TMS)	945.1	C ₆ H ₈ O ₇ Si	378150	826
21	Myristic Acid (2TMS)	950.3	C ₁₄ H ₂₇ O ₂ Si	3091296	931
22	Nε-Acetyl-Lysine (2TMS)	958.9	C ₁₂ H ₂₁ N ₃ O ₅ Si	2648154	820
23	L,5-Anhydrothreosulose (2TMS)	965.8	C ₆ H ₁₃ O ₅ Si	4981217	936
24	D-Glucose (MEOX, 2TMS)	1013.2	C ₆ H ₁₂ O ₆ Si	2561202	829
25	D-Mannitol (2TMS)	1038.6	C ₆ H ₁₄ O ₆ Si	1148235	938
26	Galactonic Acid (2TMS)	1032.2	C ₆ H ₁₂ O ₇ Si	117900	678
27	Fumaric Acid (2TMS)	1065.9	C ₄ H ₄ O ₄ Si	1706164	930
28	Methyl Galactoside (2TMS)	1074.3	C ₁₀ H ₁₉ O ₅ Si	4071300	792
29	Ferulic Acid (2TMS)	1097.5	C ₁₆ H ₁₉ O ₅ Si	19354	773
30	Monoisobutyric Acid (2TMS)	1109.0	C ₆ H ₁₃ O ₅ Si	3131958	911
31	Caffeic Acid (2TMS)	1121.9	C ₈ H ₉ O ₄ Si	595714	815
32	Dehydroabiolic Acid (2TMS)	1254.1	C ₁₅ H ₂₁ O ₅ Si	190881	809
33	Arachidonic Acid (2TMS)	1266.5	C ₁₉ H ₃₁ O ₂ Si	137383	816
34	D-Myo-Inositol Phosphate (2TMS)	1279.5	C ₁₈ H ₃₁ O ₁₀ Si	863200	909
35	3-Monolinolein (2TMS)	1340.6	C ₃₂ H ₅₉ O ₂ Si	412411	903
36	Sucrose (2TMS)	1393.4	C ₁₈ H ₃₄ O ₁₁ Si	247020	733
37	Monolinolein (2TMS)	1433.8	C ₃₂ H ₅₉ O ₂ Si	248787	871
38	1-Tocopherol (2TMS)	1534.8	C ₂₉ H ₅₀ O ₂ Si	53075	872
39	Vitamin E (2TMS)	1559.8	C ₂₉ H ₅₀ O ₂ Si	103201	942
40	Geraniol (2TMS)	1534.6	C ₁₅ H ₂₆ O ₂ Si	54376	766
41	Cholesterol (2TMS)	1080.2	C ₂₇ H ₄₆ O ₂ Si	1306018	953
42	Campesterol (2TMS)	1658.7	C ₂₈ H ₄₈ O ₂ Si	89742	740

Characterization: Spectral Similarity Searches + Accurate Mass Formulas + CI-TOFMS Data
Increased confidence in compound IDs

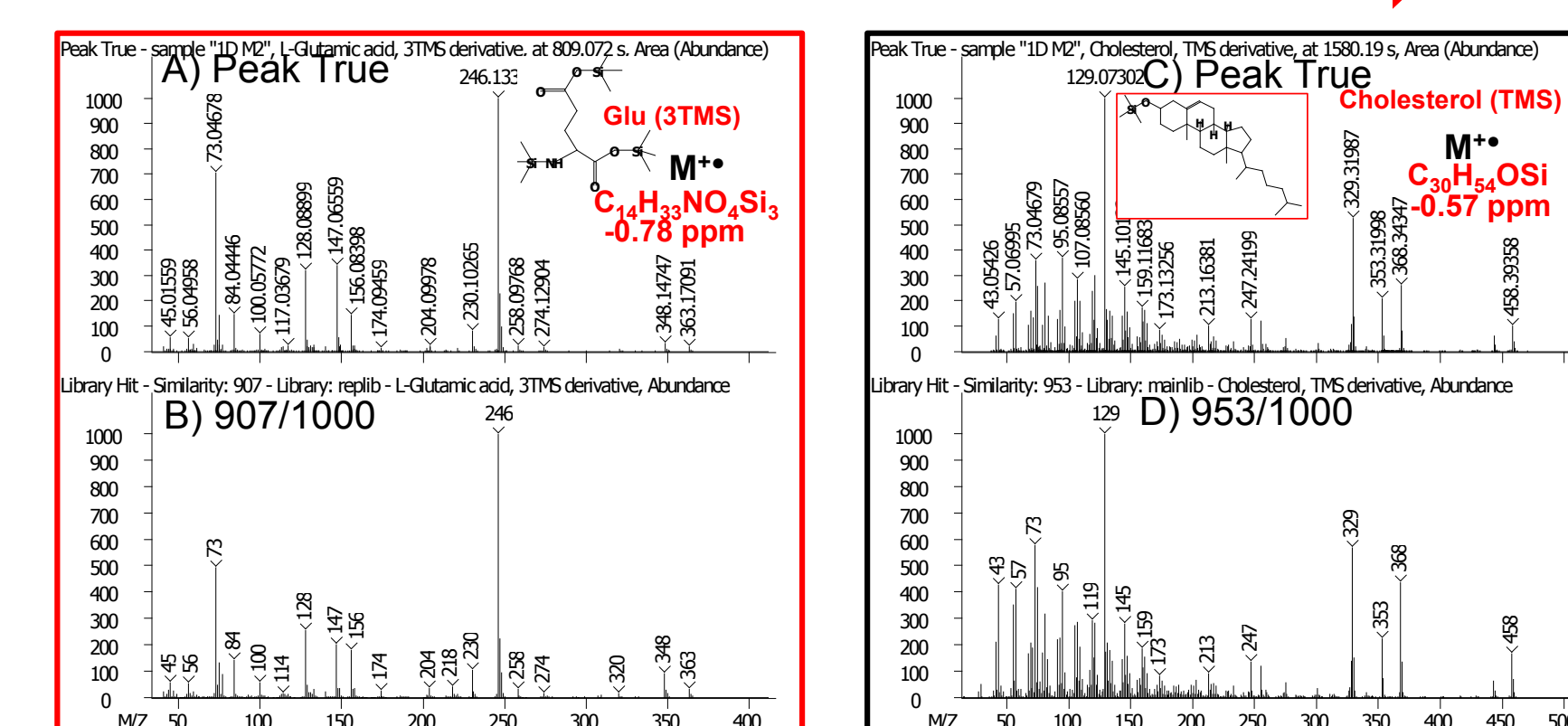


Figure 6: Peak True and library mass spectra for Glu (3TMS) and Cholesterol (TMS)

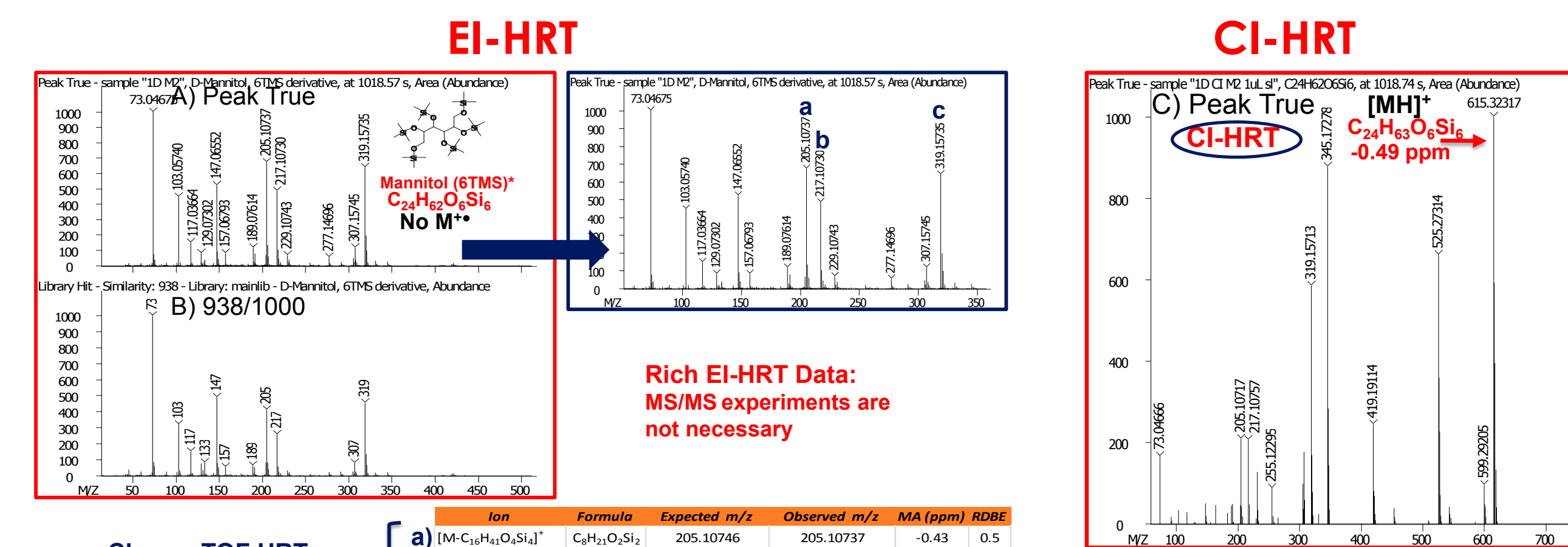


Figure 7: EI and CI-HRT Peak True and library mass spectra for mannitol (6TMS)

GCxGC-HRT Results

- Enhanced chromatographic and mass spectral resolution
- Structured chromatograms
- Removal of background interferences
- Improved compound characterization

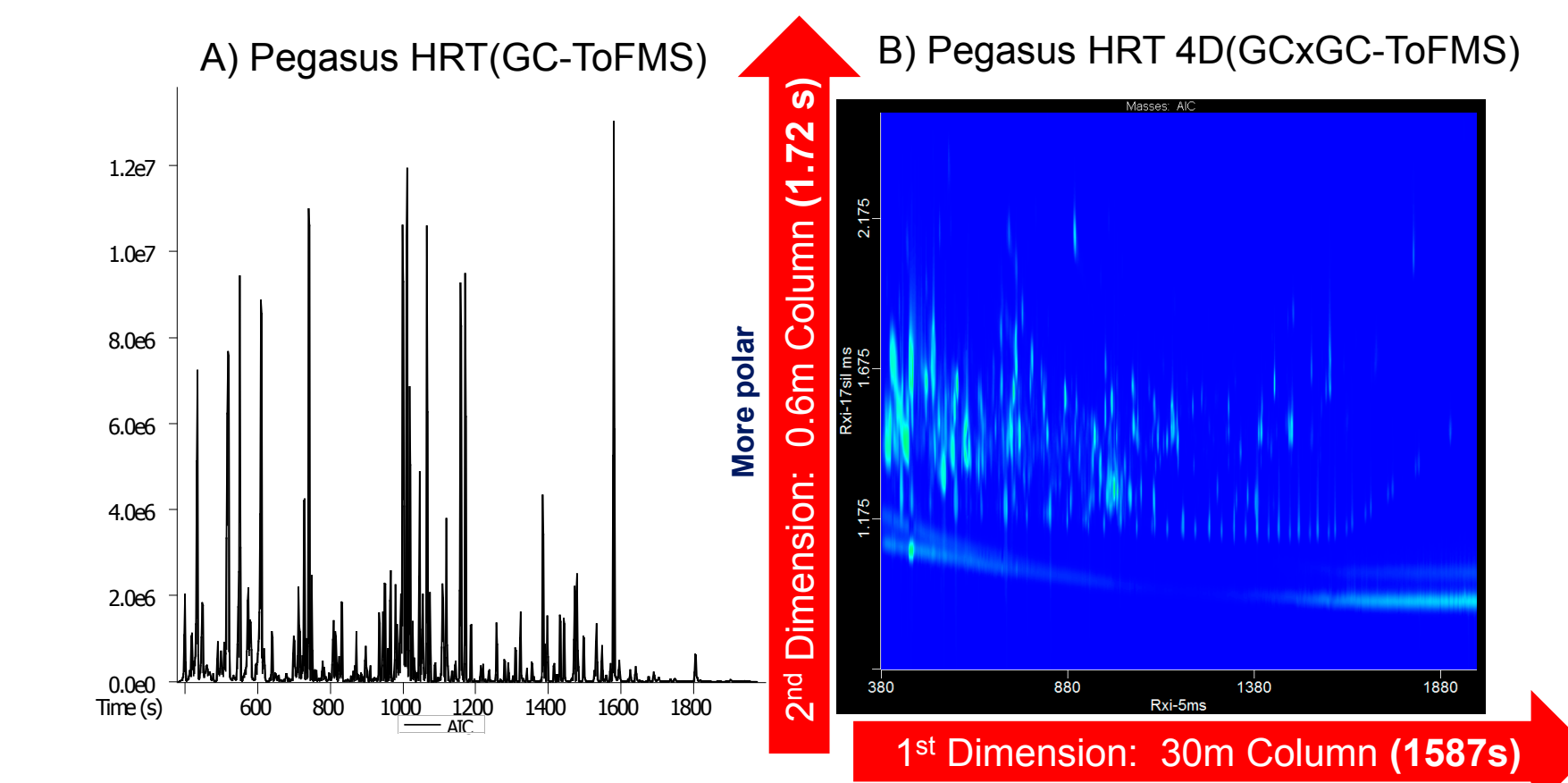


Figure 9: A) GC-HRT AIC and B) GCxGC-HRT contour plot for sample M2

Unknowns → Knowns

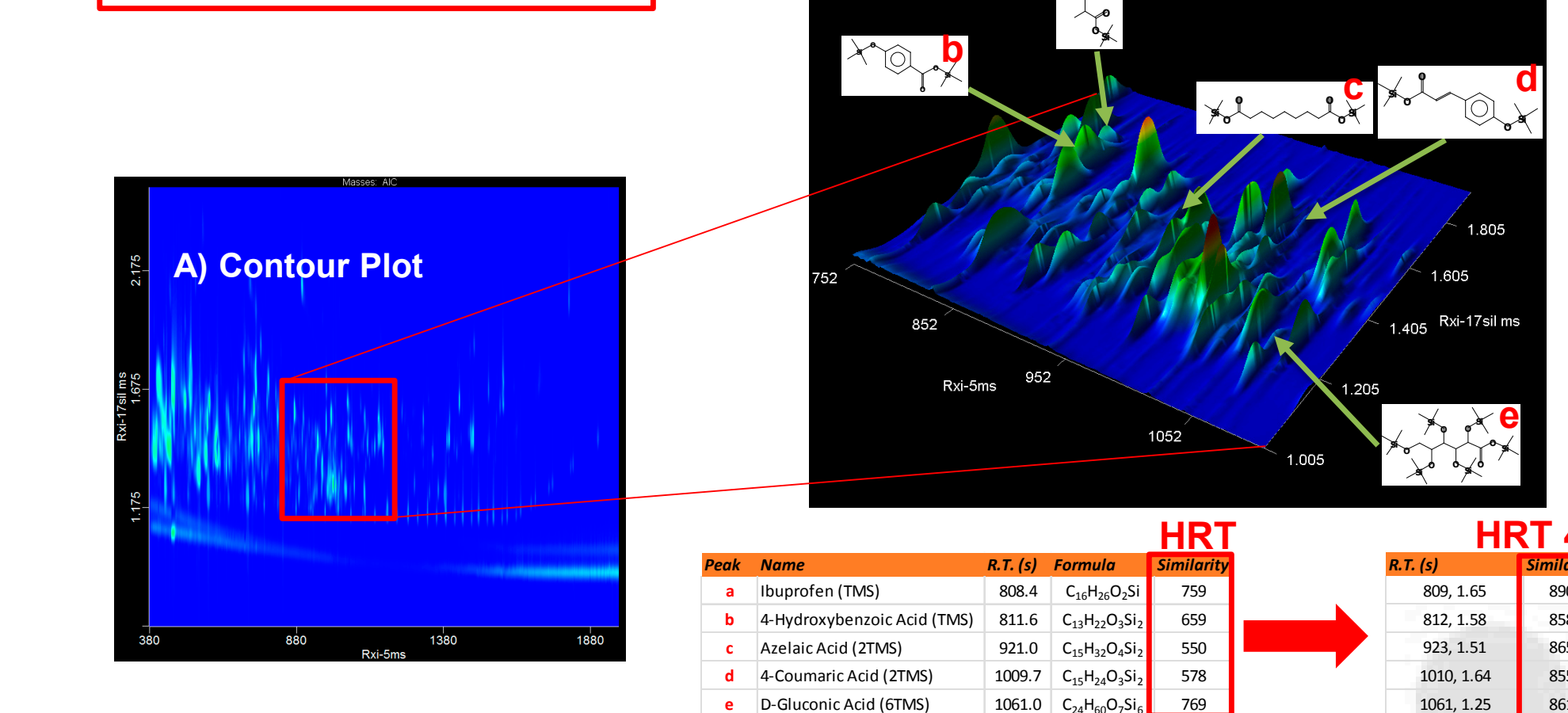


Figure 10: A) GCxGC-HRT contour and surface plots for sample M2

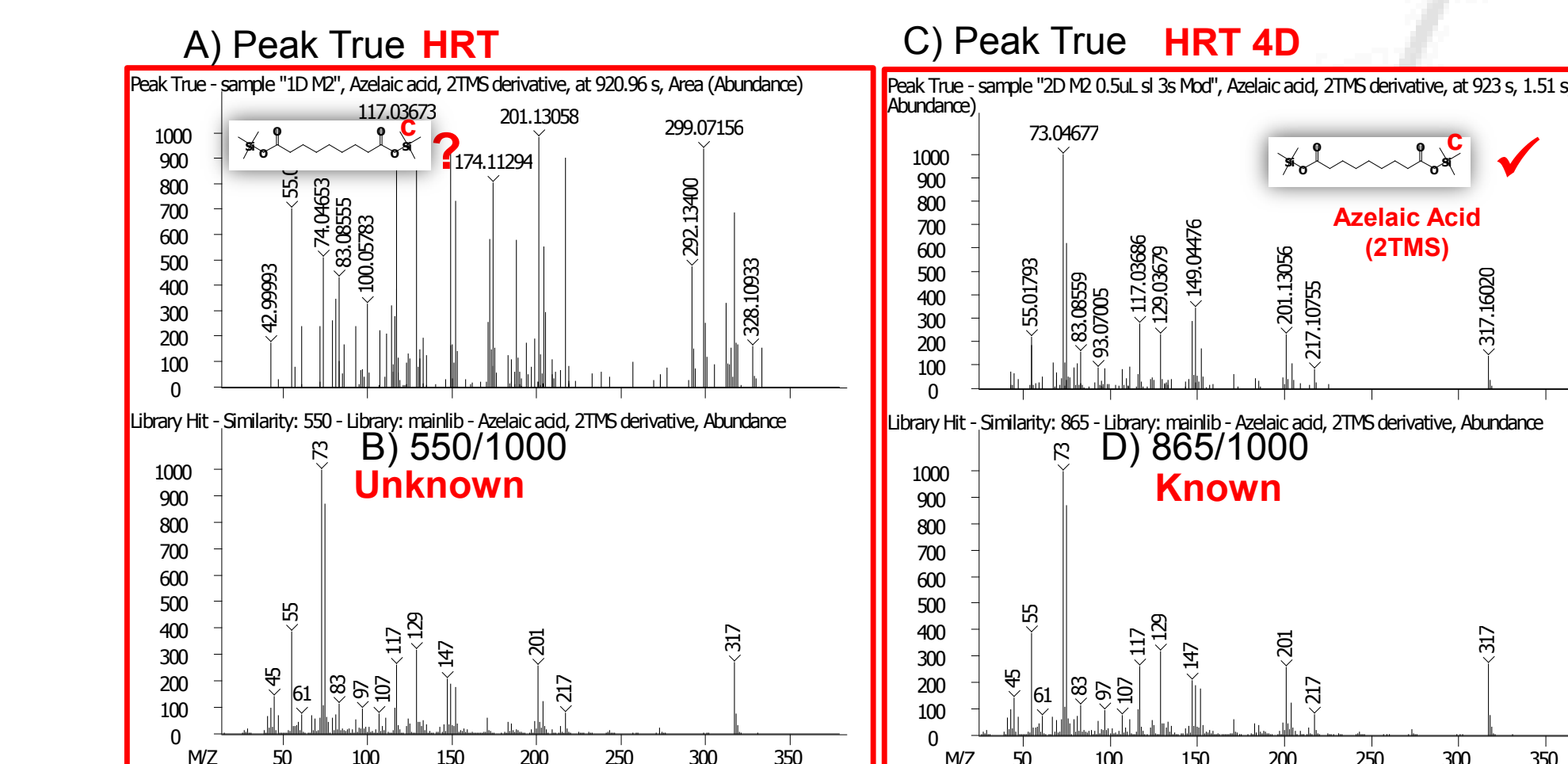
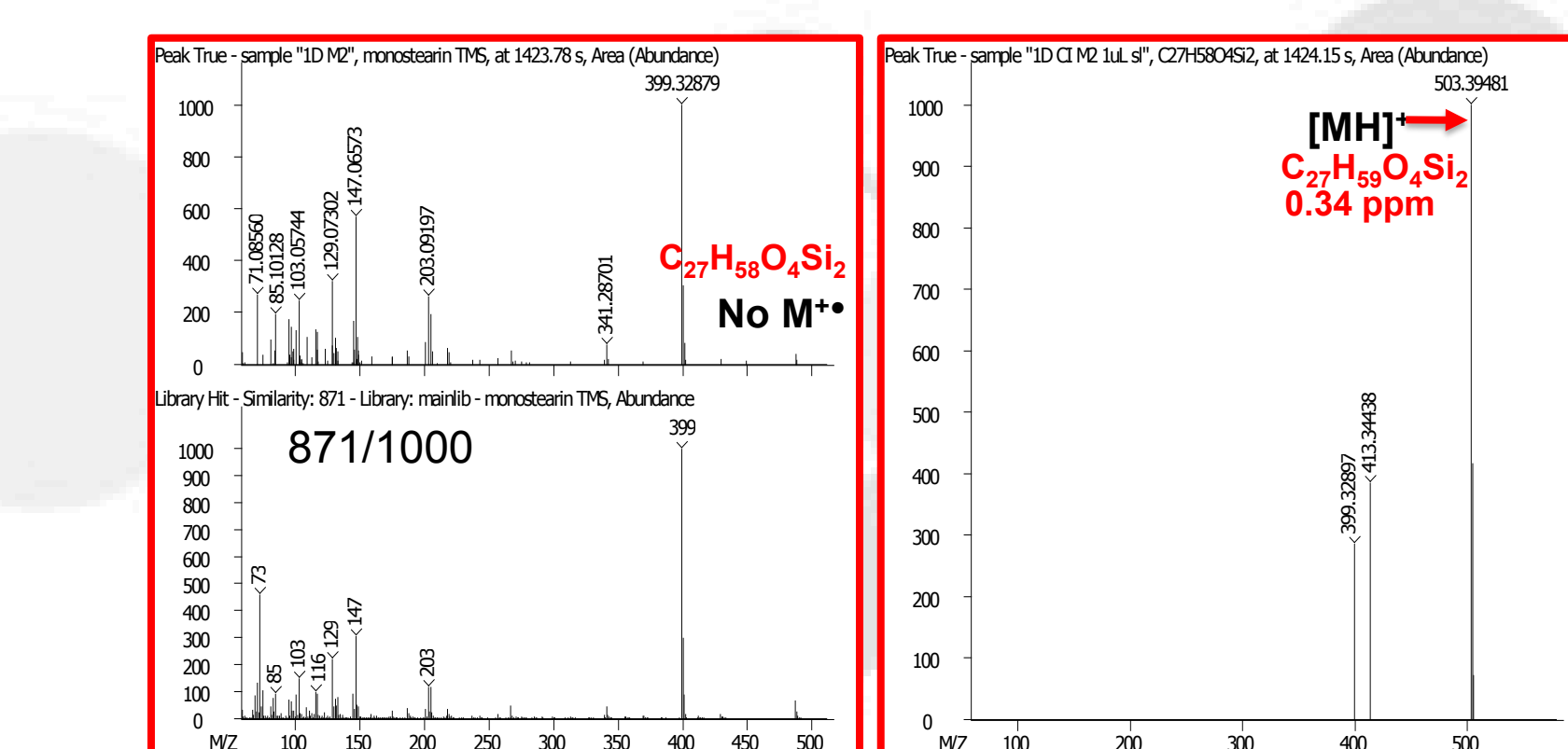


Figure 11: Comparison of HRT and HRT 4D mass spectra



Conclusion

- Quality spectra (database searches)
- Complementary EI/CI-HRT data (confirmation)
- Accurate mass (formulas)

