

Supplementary Materials: Enhanced Isotopic Ratio Outlier Analysis (IROA) peak detection and identification with ultrahigh resolution GC-Orbitrap/MS: Potential Application for Investigation of Model Organism Metabolomes

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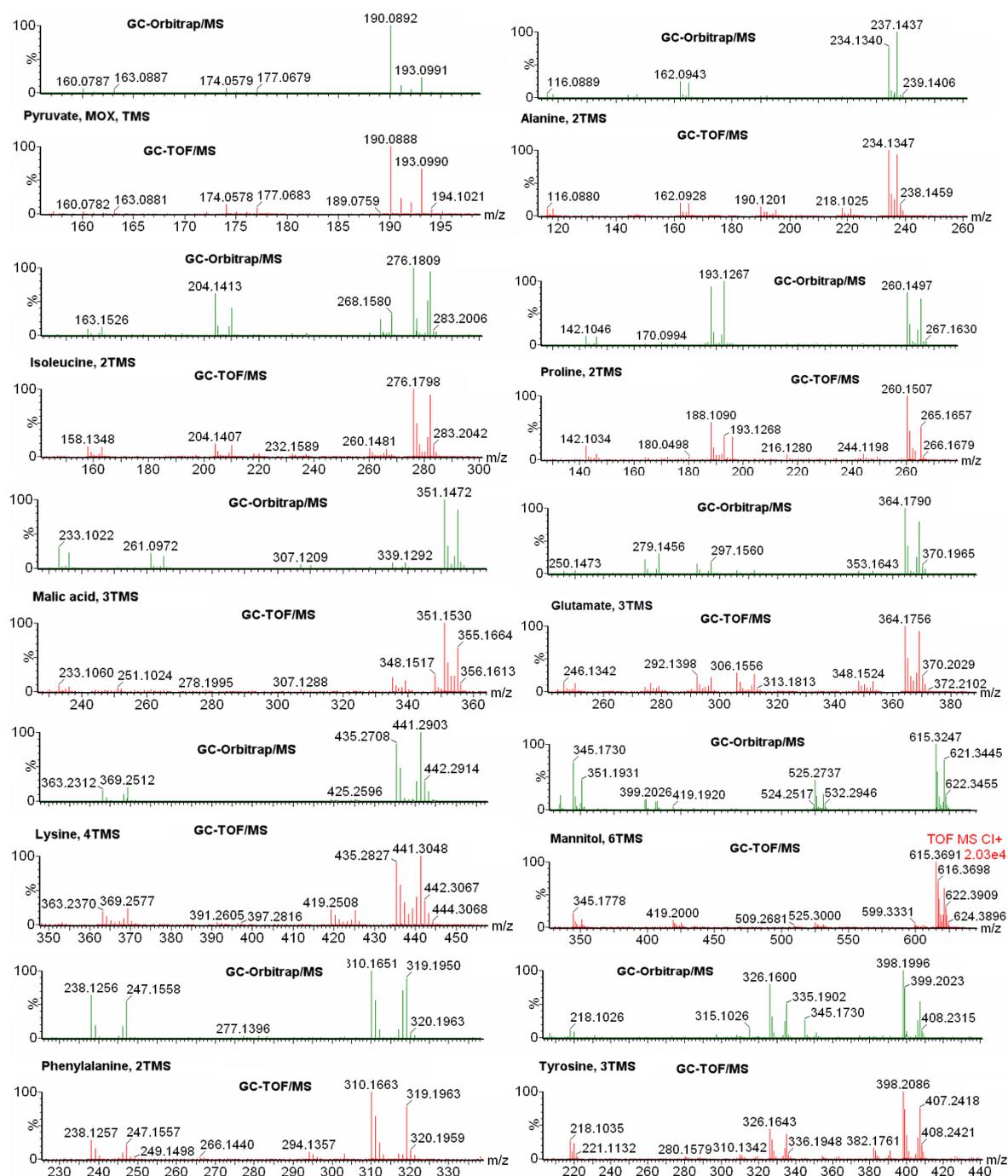


Figure S1. Mass spectra of 10 typical CI-IROA peak pairs with fragments obtained from GC-Orbitrap/MS (on the top of each panel) and GC-TOF/MS (at the bottom of each panel). The reagent gas for both is a low concentration of ammonia in methane, which for the GC-TOF/MS was 5% ammonia in methane, and 10% ammonia in methane for GC-Orbitrap/MS.

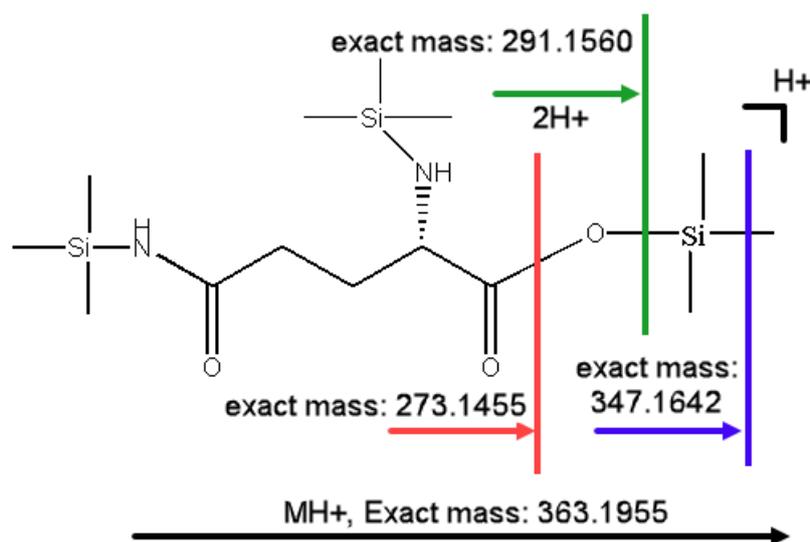


Figure S2. Elucidation of the fragmentations for tri-silylated glutamine for positive chemical ionization with methane, ammonia or the ammonia/methane mixture as the reagent gas. Pure methane as the reagent gas generated a larger intensity MH-CH₄ fragment ion ($m/z = 347.1638$ for tri-silylated glutamine), as well as a high intensity of the fragment with m/z of 273.1451, due to cleavage of an oxygen group with the silylation group attached to it. The mass spectra generated by pure ammonia and 10% ammonia in methane showed a higher relative intensity molecular ion peak (m/z 363.195), and a dominant fragment of m/z 291.155 (due to cleavage one silylation group from the molecular ion).

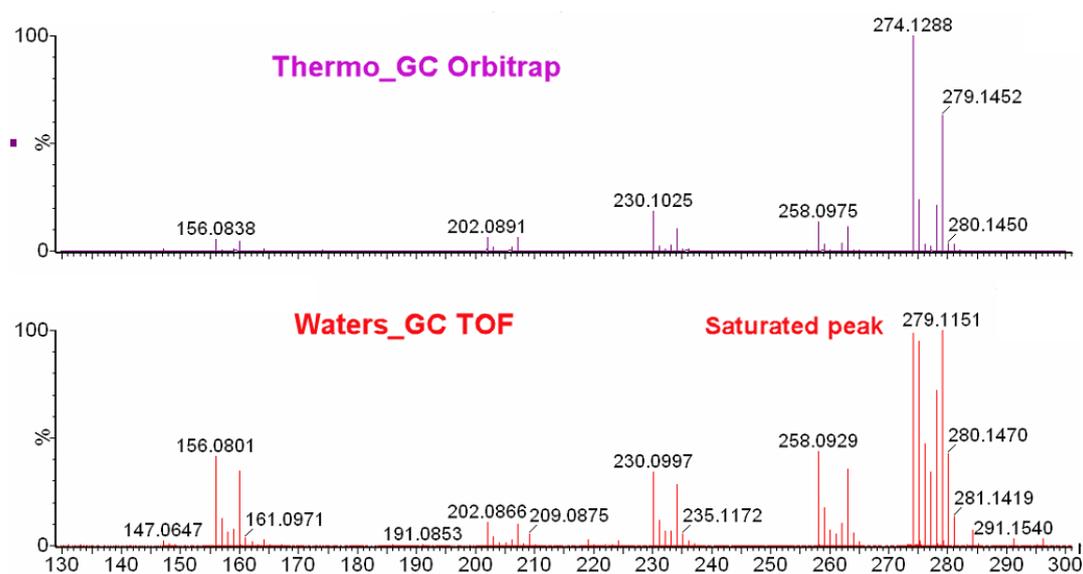


Figure S3. Demonstration of the lack of saturation for GC-Orbitrap/MS *vs.* Waters GC-TOF/MS when metabolites at high concentration (5-oxoproline) are present in the sample. To detect a greater number of lower abundance metabolites, 5 fold more extracted yeast material was used for the GC-Orbitrap/MS as compared with GC-TOF/MS. Even with a lower amount of starting material, saturation was observed in the molecular ion (m/z 274)

in GC-TOF/MS (lower) spectrum. The same IROA PCI fragments (5 carbon m/z 258/263, and m/z 202/207, and 4 carbon m/z 156/160, and m/z 230/234) were observed in both GC-TOF/MS and GC-Orbitrap/MS spectrum.

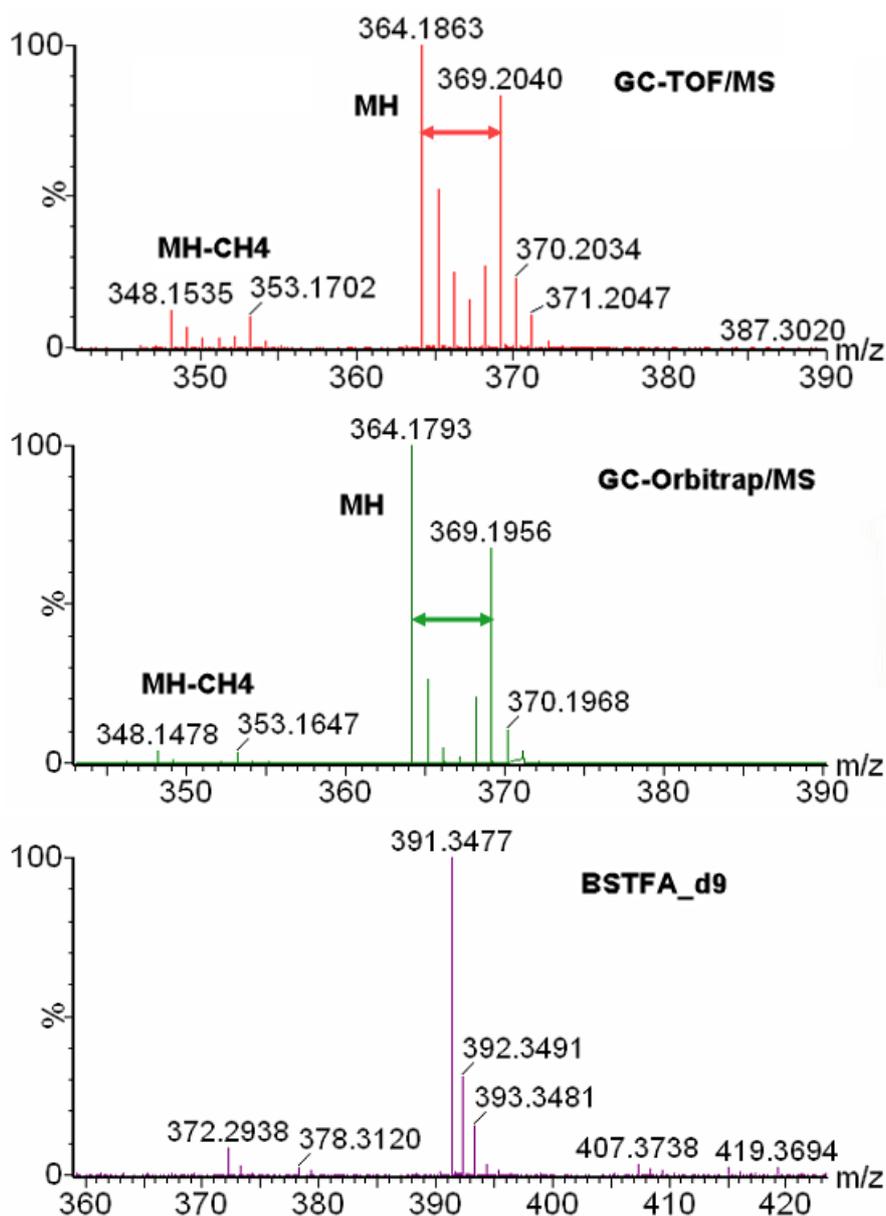


Figure S4. IROA peak pairs for tri-silylated glutamic acid detected in GC-TOF/MS (top in red color), and in GC-Orbitrap/MS (middle in green color). The $[MH]^+$ peak pairs are 364 to 369 m/z (5 carbons). BSTFA_d9 derivatization revealed 3 silylations in glutamic acid with a 27 Da difference between BSTFA_d9 and unlabeled BSTFA derivatization (m/z 364 \rightarrow m/z 391, bottom panel in purple), validating CFG predictions in Figure 2.

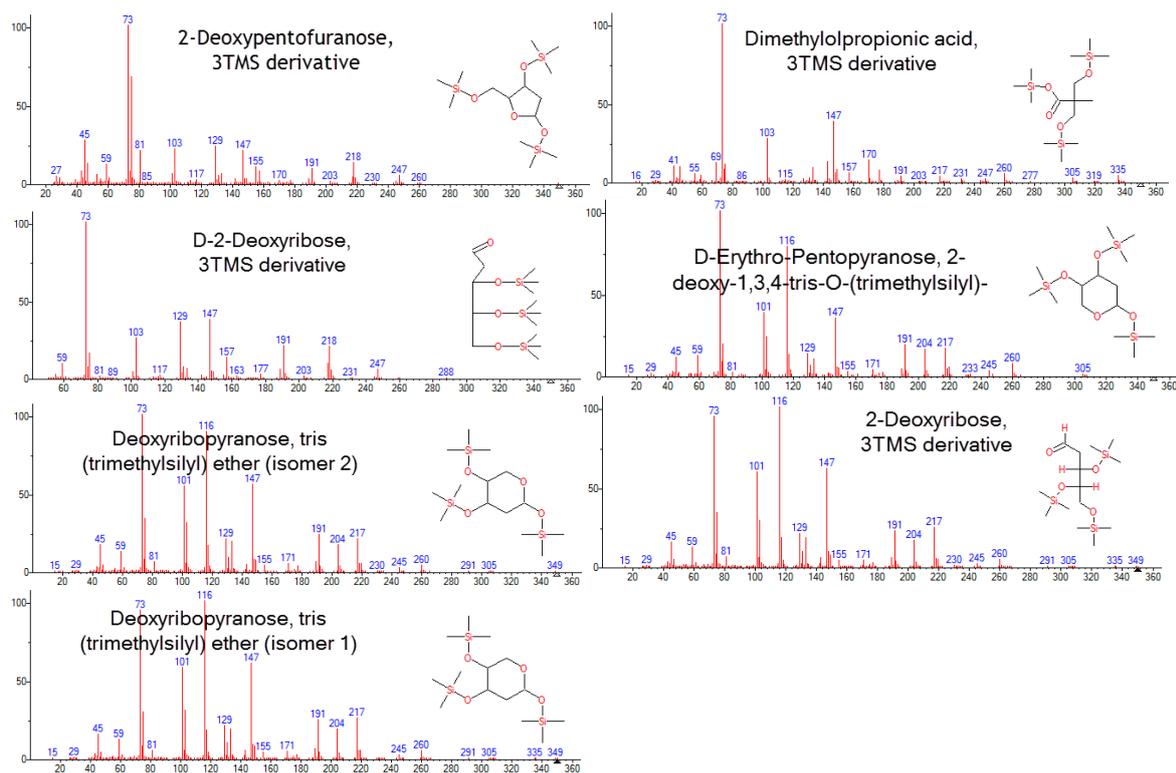


Figure S5. Representations of isomeric metabolites (seven found) with chemical formula of $C_{14}H_{34}O_4Si_3$ in the NIST library.



Figure S6. Top 10 hits using CFM-ID with the EI spectrum from the 12C IROA example (YU1130_351, see CI-IROA library in Supplemental section of reference [10]). Settings in CFM-ID: Spectra type: EI; choose a database: HMDB derivative; Parent ion mass: 350.1906; scoring function: DotProduct; with 50 ppm mass tolerance.

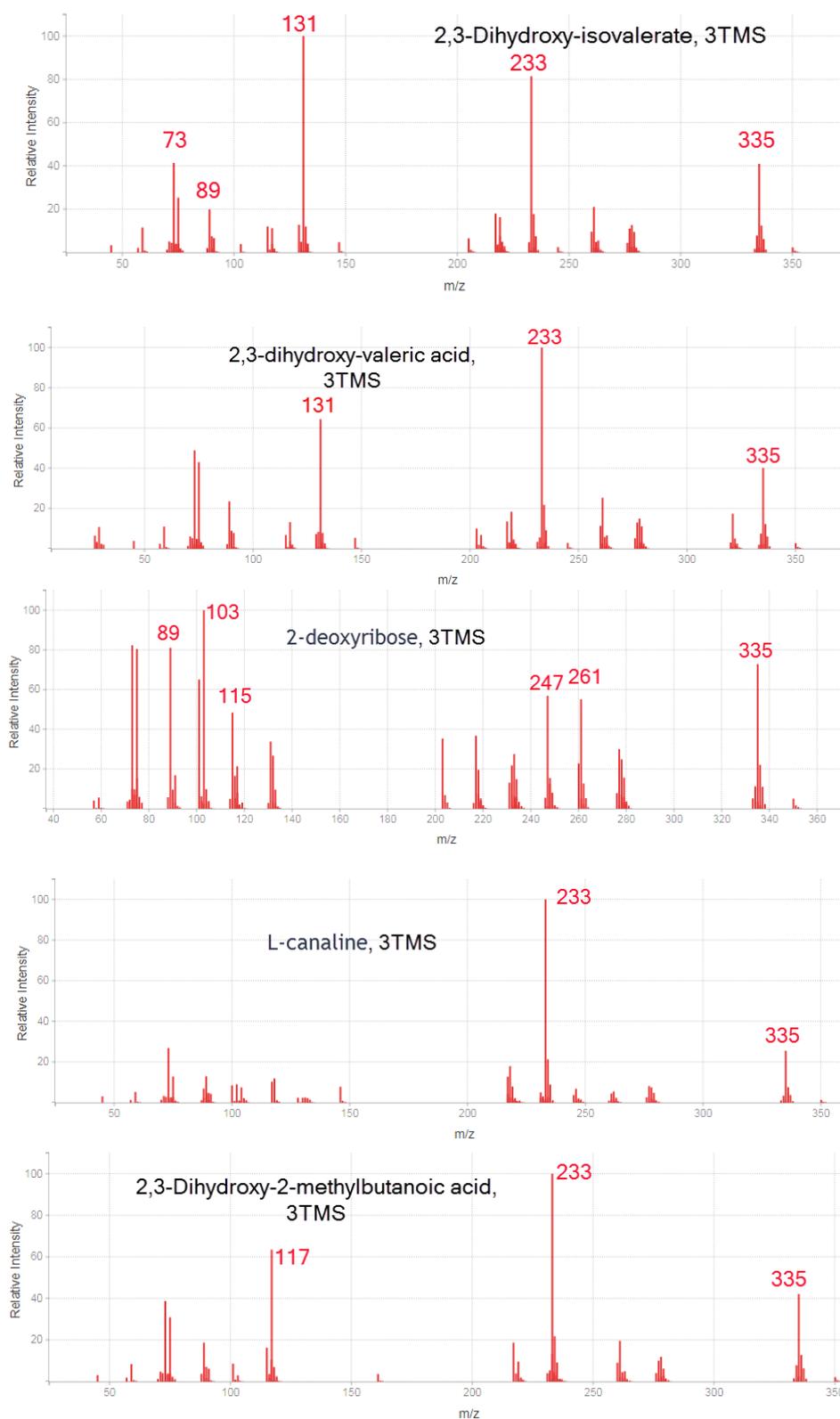


Figure S7. EI in silico fragmentation spectra of the metabolites with chemical formula of $C_{14}H_{34}O_4Si_3$ in the CFM-ID. Note that the dominant theoretical EI peak of m/z 131 is evident in the 2,3-dihydroxyisovaleric acid seen in Figure 4.