Goldschmidt 2023 Abstract
https://doi.org/10.7185/gold2023.17042

# Revealing Chemical Synthesis Pathways of Pyruvic Acid via Position-Specific Isotope Analysis (PSIA) on the GC-Orbitrap FTMS

**MR. YOUKI SATO**[1] AND KATHERINE H. FREEMAN[2]

[1]Pennsylvania State University

[2]The Pennsylvania State University

Presenting Author: yws5168@psu.edu

The distribution of carbon isotopes (i.e., $^{13}C/^{12}C$) within a given molecule reflects the carbon sources and the biological, chemical, and/or physical processes involved in its synthesis. New methods have opened access to position-specific isotope analyses, although doing so often requires method development steps that are unique for different classes of target compounds, as can be illustrated in this study of pyruvic acid. Biologically sourced pyruvic acid potentially conveys both isotopic patterns inherited from glucose along with metabolic isotope fractionation. Further, intramolecular isotope patterns are likely to differ significantly between biological pyruvate from presumably abiotic pyruvate carried by carbonaceous and chondritic meteorites.

Orbitrap-based isotope ratio mass spectrometers offer high precision and mass resolution required to differentiate and measure isotopologue ratios (molecules that are identical except for the number of isotopic substitutions) for selected ion fragments at natural isotope abundances. The capabilities of the Orbitrap to measure isotopologues can be further improved via "peak capture": trapping the analyte in a reservoir or sample loop within the GC oven and slowly releasing it to the ion source, thus increasing the amount of time a target analyte is observed [1]. The GC-Orbitrap FTMS located at Pennsylvania State University is configured with a custom sample introduction system that automates this process and allows users to measure sustained high ion intensities for over 10 minutes for nanomoles to micromoles of a specific analyte (Figure 1). Using this peak trapping configuration, isotopes values (expressed as delta values relative to the whole molecule or to a laboratory standard) of individual ion fragments could be measured at better than 2 permil precision and enabled us to observed pyruvic acid carbon positions that averaged ~1-4‰.

This method can be applied to study the intramolecular isotope fractionations that occur during the central metabolism of E. *coli*, a model heterotrophic microorganism. Pyruvic acid extracted from E. *coli* cultures grown on glucose of known intramolecular $\delta^{13}C$ can illustrate the influence of the glucose $\delta^{13}C$ vs. the kinetic isotope fractionations incurred during glycolysis while establishing an isotope pattern for the biological synthesis of pyruvate.

[1] Eiler *et al*. (2017), IJMS 422, 156-142.

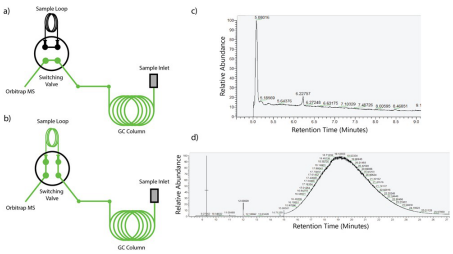


*Figure 1. Schematic of the Peak Trapper system for the Orbitrap installed at Penn State for direct injection (a) and (b) sample loop elution. (c) Representative chromatogram of analyte eluted via direct injection. (d) Representative chromatogram of an eluting analyte that has been trapped for 10 minutes, offering a high analyte signal over most of the elution period.*