The 100 isotopologue challenge: Orbitrap mass spectrometry as a means of high-dimension clumped and position-specific isotope analysis

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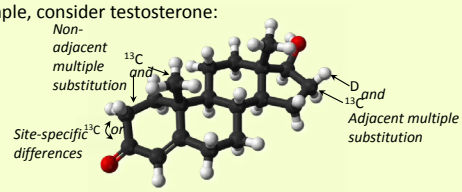
Overview

The distribution of rare isotopes (D, ^{13}C, ^{15}N, etc.) at molecular scales — site-specific effects and/or multiple substitutions, or 'clumping' — offers many potential tools in environmental chemistry, geochemistry, biochemistry and other disciplines. Existing technologies for observing molecular-scale isotopic structures (NMR, sector mass spectrometry, infrared spectroscopy) enable only a few such measurements, with limits on sample types and sizes. We present the results of an experiment to develop the Thermo Scientific™ Q Exactive™ GC Orbitrap™ instrument— an Orbitrap™ Fourier transform mass spectrometer — as a generalized tool for precise, accurate, rapid and sensitive measurements of molecular isotopic structures. Results to-date indicate this technology can achieve uncertainties in the range of 0.1-1 ‰ (part per thousand, relative, in abundance ratio) for many isotopic species, analytes and sample sizes, and is rapid enough to measure ~10-100 isotopic constraints on one compound.

Introduction

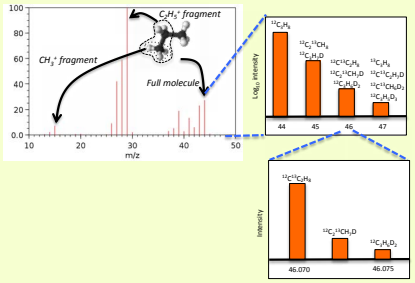
Most molecules of interest to forensics, medicine or other areas of applied chemistry exist in many isotopic forms — isotopologues that differ in their numbers and sites of isotopic substitution. For example, consider testosterone:

- *~$2x10^6$ isotopologues*
- *~10,000, restricting the list to those that are clearly abundant enough for analysis*
- *Almost all are both multiply substituted ('clumped') and 'site specific'*



Proportions of these isotopologues may vary as functions of many natural and engineered processes, and therefore an analysis that observes some large number of them could provide exceptionally detailed constraints on molecular origins.

Mass spectrometry is the only technology that currently seems capable of quantifying large numbers of site-specific *and* clumped isotope species in small samples (sub micromol) of diverse compounds. This is because peaks in a molecule's mass spectrum often come from some known (or knowable) combination of molecular sites, and such peaks exist in numerous isotopic forms.



Even the mass spectrum of something as simple as propane contains families of peaks that sub-sample different portions of the molecule, and each peak exists in several singly- and multiply-substituted forms, potentially offering dozens of constraints on proportions of site-specific and 'clumped' isotopologues.

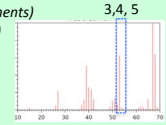
However, established mass spectrometric approaches lack the mass resolution to study any but the smallest molecules (~50-70 amu or less), and are far too slow to constrain more than a handful of isotopologues per molecule.

Methods I: Mass Spectrometry

Here we explore Fourier transform mass spectrometry (FTMS) as a solution to this problem, using the Q Exactive GC system— a recent innovation on Orbitrap-based FTMS, where analytes are delivered by gas flow, ions are generated by electron impact, mass-range selection is made with the 'Advanced Quadrupole Technology' (AQT), and mass analysis is made using the Orbitrap detection. Peaks of interest may also be subjected to an MS-MS experiment using the HCD collision cell. This system routinely achieves mass resolutions of 10^5, and enables rapid, automated analysis of large numbers of peaks across a large mass range.

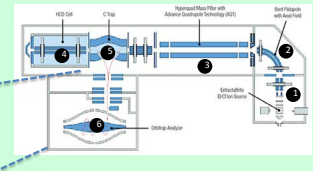
5: The selected ions (or their collisional fragments) are stored in the C trap (repeat as desired)

3,4, 5

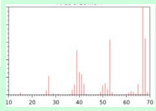


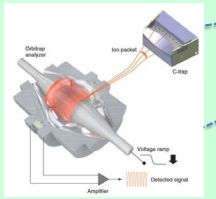
4: (optional): Selected masses may be passed to the HCD cell and subjected to fragmentation by energetic collisions

3: The AQT is used to select a mass range of interest (≥0.4 amu range, with resolution of ~1000)



2: All ions are extracted, transferred through the bent flatapole, and collisionally cooled



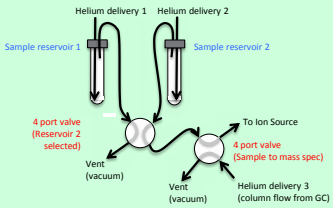


6: After steps 1-5 are performed 1 or more times, all ions accumulated in the C trap are injected into the Orbitrap and observed

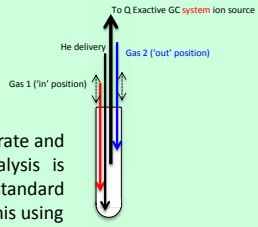
1: Analyte is introduced and ionized by electron bombardment

Methods II: Sample Introduction

(3) Two purged reservoirs — room temperature liquids and solids

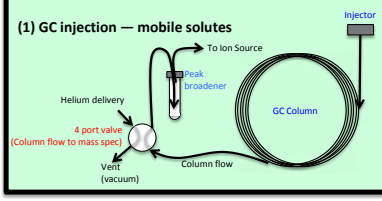


(2) Conflo™ open split — room temperature gases



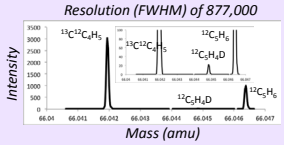
A key requirement of accurate and precise isotope ratio analysis is highly controlled sample/standard comparison. We achieve this using any of three systems we have adapted to the Q Exactive GC instrument: (1) A GC column for analysis of eluted solute peaks, adapted to send sample peaks through a reservoir that broadens them, increasing the time a peak is observed; (2) an open split for analysis of room-temperature gases; and (3) a pair of reservoirs that can stably deliver sample or standard to the ion source for minutes to hours.

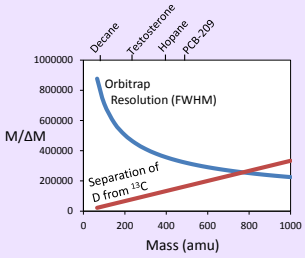
(1) GC injection — mobile solutes



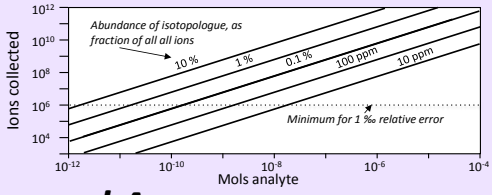
Results I: Resolution and Sensitivity

A select portion of the ethyl benzene mass spectrum

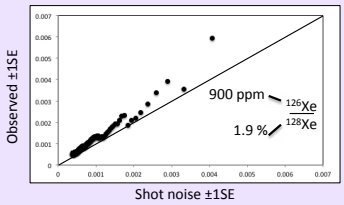




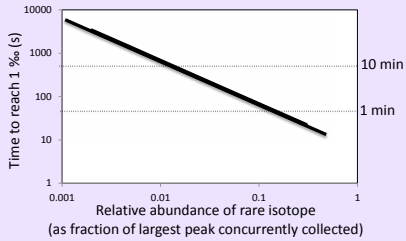
Mass resolution of the Orbitrap mass analyzer depends on acquisition time and analyte mass, but generally greatly exceeds anything possible by the sector instruments used for isotope ratio analysis, and is clearly sufficient to separate common isobars (upper left). Separation of isotopologues of organics and their fragment ions is robust up to ~500 amu (lower left). Sensitivity varies with analyte and tuning, but is generally on the order of 10^5 molecules per ion, with baselines of a few ions per second, implying sample sizes $<10^{-6}$ mols for many isotopologues (please see below).



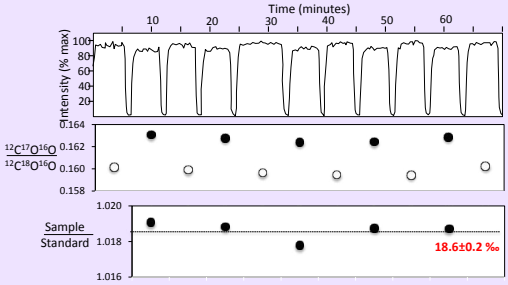
Results II: Precision and Accuracy



1 ‰ in 3 minutes; 0.4 ‰ in 12.5 minutes

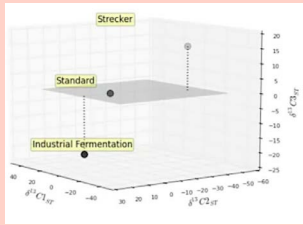


External errors of Orbitrap analyses of isotope ratios are generally limited by shot-noise errors alone, down to limits of ~0.1-1‰ (upper left). This fact implies that limits of precision are mostly controlled by relative peak intensities and time (upper right); the fastest measurements generally look at only a portion of the mass spectrum at one time. Sample/standard bracketing (lower right) permits standardization with accuracy similar to precision (tenths of ‰).



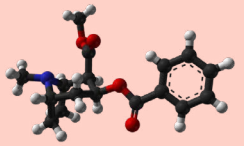
Example of Use

Representative analyses of the site-specific ^{13}C structures of three amino acids from different sources. C1, C2 and C3 are carboxyl, amine and methyl carbons, respectively. All reported $\delta^{13}C$ values are relative to an arbitrary standard (VWR stock L alanine). All three materials span a narrow range in bulk $\delta^{13}C_{PDB}$ but they differ strongly in site specific 'finger print'.

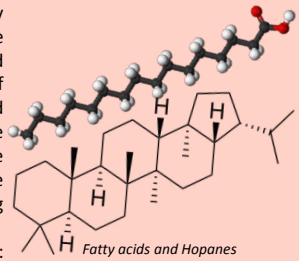


Fields of Application

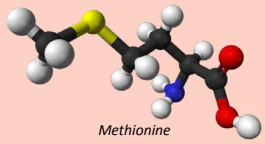
- **Forensics**: Isotopic 'fingerprinting' that includes ~10-100 independent measures of site-specific and multiple substitutions will greatly advance the specificity of molecular sources in forensic studies of compounds relevant to environmental pollution, crime, performance enhancing drugs, and national security.
- **Biogeochemistry**: Proportions of multiply substituted and site-specific isotopologues are known to constrain temperatures and mechanisms of molecular synthesis, identities of source substrates, and mechanisms and amounts of molecular destruction or loss. These capabilities will open new windows on the origins of ancient biomarkers, paleoclimate records based on organic compounds, among other uses.
- **Personalized medicine and metabolomics**: High-dimensionality, sensitive measurements of molecular isotopic structure will enable constraints on metabolite and drug budgets normally obtained from isotopic labeling experiments, but using the naturally occurring isotopes.



Cocaine



Fatty acids and Hopanes



Methionine

Conclusions

Orbitrap-based FT isotope ratio mass spectrometry (FT-IRMS) has several strengths: Mass resolution of substitutions involving isotopes of H, C, N, O and S at molecular or fragment masses ≤500 amu; the speed and mass range to measure many such species per sample in minutes to hours; the sensitivity for quantitative analysis of sub-micro-molar samples, or doubly and triply substituted species at their trace natural abundances; the ability to receive diverse analytes in several forms and delivery systems; and precision and accuracy comparable to existing technologies. These capabilities will enable a large number of new applications in biological, chemical and natural sciences.

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