

Possibilities and challenges of ESI-Orbitrap-MS as a tool for isotopocule analysis on organic molecules

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High precision natural abundance isotope ratio analysis of complex organic molecules has been an important tool in understanding planetary processes throughout the last decades. State of the art workflows often require the conversion of complex organic molecules to low molecular weight gases to perform a high precision isotope quantification using sector field MS. Recently, the high-resolution accurate-mass of Thermo Scientific™ Orbitrap™ MS has been shown to enable simultaneous, high precision analysis of intact isotopocules with isotopic substitutions from multiple elements, which bears new potential for gathering intramolecular isotopic information.

Here we highlight improvements in ESI-Orbitrap IRMS for the analysis of organic molecules using ¹³C, ²H, ¹⁸O and ¹⁵N substituted isotopocules of caffeine and vanillin as a model. An exemplary mass spectrum of caffeine acquired with a resolution of 240,000 at 200 *m/z* is shown in figure 1. Orbitrap results are compared and benchmarked against international reference materials and results obtained by sector field MS revealing accuracies and precisions down to <0.5 ‰ for carbon, 2 ‰ for oxygen, 10 ‰ for hydrogen and 1 ‰ for nitrogen isotopocule ratios. The presented data will show how challenges in Orbitrap-IRMS for complex organic molecules can be overcome by dedicated referencing and calibration procedures, highlighting the need for a wider availability of isotopically characterized reference materials. Performing efficient sample/standard comparison sets requirements on the sample introduction techniques. While many different techniques have been developed for reference introduction in the gas phase, liquid sample and reference introduction approaches still lack the efficiency and robustness of their gaseous counterparts. To this end, we have explored novel sample introduction techniques using different autosampler configurations and online liquid chromatography for better automation and matrix tolerance specifically targeting high precision natural abundance isotopocule ratio quantification in complex organic mixtures. Preliminary data of caffeine isotopocule ratios in six different beverages analyzed by online coupling of reversed-phase liquid chromatography to ESI-Orbitrap IRMS is shown in figure 2.

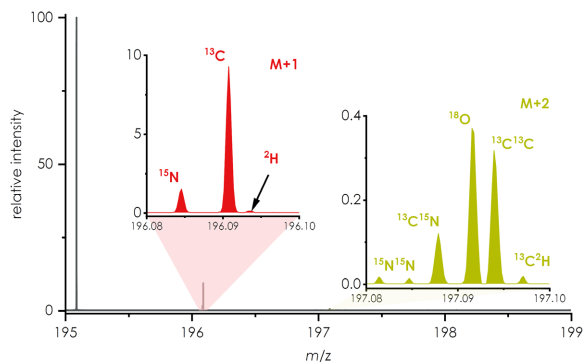


Figure 1: Caffeine mass spectrum acquired at 240,000 resolution at 200 *m/z*. Peaks are labeled based on the heavy isotope substitution of the according caffeine isotopocule.

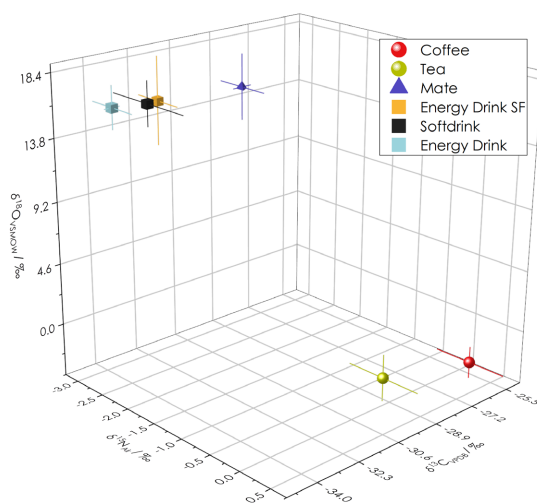


Figure 2: Three-dimensional plot of caffeine isotopocule ratios in six different beverages analyzed by online coupling of reversed-phase liquid chromatography to ESI-Orbitrap IRMS.