

Position-Specific Isotope Analysis of Serine Using ESI-Orbitrap

Martin Elsner^{1*} & Leonhard Precht¹

¹ Chair of Analytical Chemistry and Water Chemistry, Technical University of Munich

*Presenting Author Email: m.elsner@tum.de

Serine is a proteinogenic amino acid and an essential nutrient for the bacterium *Legionella pneumophila*, which relies on serine as its primary carbon and energy source. Consequently, variations in the metabolism of *L. pneumophila* are expected to be reflected in the carbon isotope ratios of serine within its proteins.

Traditional methods for serine isotope ratio analysis have relied on bulk isotope analysis of pure serine using EA-IRMS, with position-specific analysis of the carboxy carbon achieved through ninhydrin reaction and subsequent CO₂ measurement. Comprehensive position-specific isotope analysis of serine has previously required extensive derivatization for GC-Orbitrap analysis.

We have explored a novel method for position-specific isotope analysis of serine using ESI-Orbitrap, eliminating the need for derivatization. This method combines whole-molecule isotope ratio measurements with those obtained from various fragments generated in a collision cell, in both positive and negative ESI modes.

To support this method, we have prepared homogeneous position-specific carbon isotope standards for all carbon atoms in serine, resulting in eight distinct standards with varying ¹³C/¹²C isotope ratios. These standards enable precise calibration and referencing of serine isotope measurements on the Orbitrap and facilitate inter-laboratory comparisons of measured isotope ratios.

In the future we want to conduct metabolic studies of *L. pneumophila* by monitoring the change of serine carbon isotope ratios without the need of expensive ¹³C-labeled serine in the growth medium. This might also allow metabolic studies of *L. pneumophila* samples directly from the environment.