

Site-Specific $\delta^{13}\text{C}$, D and Clumped Isotope Analysis of Steroidal Compounds for Forensic and Geochemical Applications

LUBNA SHAWAR¹, THOMAS PIPER² AND JOHN EILER¹

¹California Institute of Technology

²German Sport University Cologne

Presenting Author: Lubnas@Caltech.edu

Steroidal compounds are crucial biomolecules in nature, governing diverse biological functions and serving as indicators of organic matter origin, depositional environmental history, and facilitating geological correlations in the petroleum geochemistry (Moldowan et al., 1985; Volkman, 2003). Nevertheless, different organisms can generate identical steroidal compounds, so confident identifications of sources can be enhanced by stable isotope analysis. Prior studies have demonstrated the utility of molecular-average $\delta^{13}\text{C}$ to improve interpretations of the phylogenetic and environmental origins of steroidal compounds (Freeman et al., 1990). However, there are challenges and limitations associated with interpretation of $\delta^{13}\text{C}$ values of steroids in geochemical contexts, including post-depositional alteration and the influence of different abiotic and biotic processes. In addition, $\delta^{13}\text{C}$ values of steroidal compounds from different sources might overlap (Piper and Thevis, 2022).

We will present results of a novel Orbitrap-based analytical method for measuring multiple stable-isotope properties of steroidal compounds, including the intramolecular distributions of single and multiple ^{13}C and D substitutions, with the aim of providing more reliable constraints of sources, environments and alteration histories. Unlike traditional bulk carbon isotope analysis, site-specific and multiply substituted carbon isotope analysis focuses on specific carbon positions or groups of positions within organic molecules, enabling detection of variations in carbon cycling, metabolic pathways, and microbial processes that may not be evident from bulk measurements alone. A first proof of concept study focuses on forensic discrimination of isotopic structures of natural and synthetic steroids in human subjects for the purpose of sports doping applications. Preliminary results reveal differences in the measured site-specific ^{13}C , D and clumped isotope of various isotopologues derived from natural and synthetic steroids despite the similarity in their molecular average isotope values.

References

Freeman, K.H., et al., 1990. Evidence from carbon isotope measurements for diverse origins of sedimentary hydrocarbons. *Nature* 343, 254-256.

Moldowan, J.M., et al., 1985. Relationship Between Petroleum Composition and Depositional Environment of Petroleum Source Rocks. *AAPG Bull.* 69, 1255-1268.

Piper, T., Thevis, M., 2022. Investigations in carbon isotope ratios of seized testosterone and boldenone preparations. *Drug Test. Anal.* 14, 514-518.