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Sterol Biomarkers for Paleoenvironmental and Anthropogenic Tracing in Speleothems

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Speleothems, renowned for their potential as continuous paleoenvironmental archives spanning thousands of years, are particularly valuable due to reliable age determination via the 230Th/U230Th/U-method. The closed-system nature of cave environments and the chemical stability of speleothems allow for the preservation and analysis of organic substances alongside traditional proxies such as stable isotopes and trace elements. Among organic compounds, sterols emerge as promising biomarkers owing to their chemical stability in oxygen-limited environments, and distinct origins from plants, animals, and microbial processes.

Cholesterol and sitosterol, representing sterols derived from animals and plants, respectively, are precursors to stanols, which are microbially reduced sterols, often traceable to faecal inputs. Notably, coprostanol serves as a key marker for human activity due to its predominance in human faeces. Despite the widespread application of sterol-based biomarkers in soil and sediment studies, their use in speleothem research remains nascent, largely due to the challenges posed by the complex mineral matrix and low concentrations of organic analytes.

To overcome these obstacles, a method combining stir bar sorptive extraction (SBSE) with a polydimethylsiloxane (PDMS) phase was developed, following acid dissolution of speleothem samples. Subsequent analysis was performed using high-performance liquid chromatography coupled with atmospheric pressure chemical ionization high-resolution orbitrap mass spectrometry (HPLC-APCI-HRMS), which provides exceptional resolution and sensitivity. This novel methodology not only enhances the extraction and analysis of sterols from speleothems but also establishes a pathway for expanding their use in paleoenvironmental and anthropogenic reconstructions.