

Bacteriohopanepolyols (BHPs) are characterized by a large structural diversity. The occurrence of isomers among composite BHPs in environmental samples has received limited attention. Here, a novel chromatographic method was developed to separate BHP isomers. Lipid extracts from marine core-top sediments were analyzed using ultra-high resolution liquid chromatography/triple-quadrupole mass spectrometry in selective reaction monitoring mode (UHPLC/QqQ/SRM). BHP isomers were separated isocratically on three serial Phenomenex Kinetex C₁₈ columns. The new UHPLC/QqQ/SRM method successfully resolves up to six structural isomers of the most abundant BHPs detected consistently in the investigated marine sediment samples. Various novel BHP isomers were consistently found in marine sedimentary extracts, but their relative abundance changed significantly among the samples seemingly related to environmental conditions. Comparison of BHP abundances obtained with previously published methods implies that robust quantification of absolute and relative BHP abundances relies on good chromatographic separation of structural isomers.