**CHIRAL SEPARATION, CHROMATOGRAPHIC MODELLING, SPATIAL DISTRIBUTION AND PHYLOGENETIC PATTERNS OF USNIC ACID ENANTIOMERS**

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Usnic acids (UA) constitute the major secondary metabolite in many lichen taxa, but the contents of UA enantiomers different lichen lineages are not well known. This is mainly due to the lack a valid chromatographic method. We developed and validated a chiral chromatographic method for quantification of usnic acid enantiomers in lichens. In our datasets, UA contents vary from 0.5 to 7.8%, and usually only one enantiomer is produced as the major form. Molecular simulation and modelling were performed to elucidate separation mechanisms. Fluorescent microscopy and mass spectrometry imaging were applied to explore the spatial distribution of usnic acids in lichen cross-sections, which revealed an uneven distribution on thallus surfaces. Quantitative data were mapped onto lichenized fungal tree, and a phylogenetic clustering of usnic acid enantiomer production was found.