ABSTRACT

Lipidomics is a rapidly expanding research field that has captured extensive attention worldwide in the past few years due to the increasing awareness of the crucial roles of lipids in biological systems. The aim of lipidomics is to comprehensively analyze all lipids, to study their structure, biological function within the cell as well as interactions of lipids with other molecules. A combination of advanced analytical techniques, such as extraction, chromatography and mass spectrometry, is an effective tool for studying all aspects of lipidomics. This dissertation thesis is based on two journal publications and presents application of analytical strategies based on chromatography and mass spectrometry for investigation and characterization of new lipid classes of vernix caseosa.

Firstly, the applicability of nonaqueous reversed-phase liquid chromatography atmospheric pressure ionization tandem mass spectrometry (LC-APCI-MS^2) for structural characterization of cholesteryl esters of ω-(*O*-acyl)-hydroxy fatty acids (Chl-ωOAHFAs) in vernix caseosa was investigated. For this purpose, a TLC chromatography method for the isolation of neutral Chl-ωOAHFAs from vernix caseosa was developed. Their general structure was established using multi-step mass spectrometric approach requiring transesterification and derivatization steps. To get the structural information of Chl-ωOAHFAs individual species, a mass spectrometric method using controlled thermal decomposition and data-dependent fragmentation was carefully optimized. About three hundred molecular species of Chl-ωOAHFAs were identified and quantified.

Secondly, while exploring vernix caseosa, a structurally related subclass of Chl-ωOAHFAs lipids, namely ω-(*O*-acyl)-hydroxy fatty acids (ωOAHFAs), was

discovered. OAHFAs were isolated from a sample of vernix caseosa by an optimized 2-step TLC on silica gel. Using high performance liquid chromatography coupled-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS^2), we identified and quantified a group of more than 400 species. Both Chl-ωOAHFAs and ωOAHFAs has been detected in other biological materials, such as human meibum or skin. However, when investigating the structure of ωOAHFAs we revealed presence of α-isomers of OAHFAs. Intact αOAHFAs has never been detected before in any biological material.