DEVELOPMENT OF TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY METHOD FOR ANALYSIS OF OLIGOSACCHARIDES FROM HYDROPHOBIC HYALURONAN DERIVATIVES

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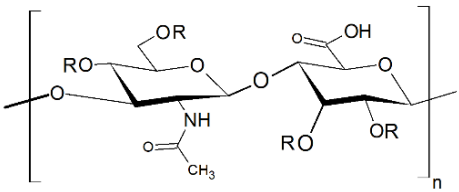
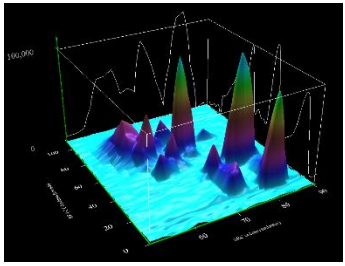
Derivatives of hyaluronan with hydrophobic functional groups are promising macromolecules for many possible biomedical applications.[1] For complete characterization of novel derivatives not only essential parameters are needed, such as a degree of substitution, molar mass distribution and polydispersity, but also distribution of substituents along the molecule chain[2] is indispensable for proper assessment of structure-property relationship of these polymers. A method, that utilizes two-dimensional liquid chromatography hyphenated with mass spectrometry for separation of oligosaccharides derived from hyaluronan derivatives, was developed. Oligosaccharides can be obtained by enzymatic degradation of polymeric derivatives with hyaluronidases (e.g. from *Streptococcus Pneumoniae*)[3] and because of digestion they differ in a length (a number of hyaluronan units) and in a number of bound substituents. Therefore, the combination of size-exclusion chromatography in the first dimension and reversed-phase LC in the second dimension was used to successfully separate complicated mixtures of oligosaccharides by size and polarity at the same time. Also, the active solvent modulation was used while connecting two modes of separation which can suppress a “breakthrough” effect[4] and further help with optimizing the separation.



R = H or CH_3-$(CH_2)_{10}$-CO

lauroyl derivative of HA

enzyme
SpHyl



separated oligosaccharides

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