Deciphering Salt and Solvent Effects in the Chromatographic Separation of Heparan Sulfate Disaccharides

Tóth G[1,2], Vékey K[1], Horváth V[2,3], Drahos L[1] and Turiák L[1]*

[1] *MS Proteomics Research Group, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Magyar tudósok körútja 2., H-1117 Budapest, Hungary*

[2]*Department of Inorganic and Analytical Chemistry, Budapest University of Technology and Economics, Szent Gellért tér 4., H-1111 Budapest, Hungary*

[3]*MTA-BME Computation Driven Chemistry Research Group, Szent Gellért tér 4., H-1111 Budapest, Hungary*

Heparan sulfate (HS) belongs to the class of glycosaminoglycans, it is a polysaccharide consisting of repeating disaccharide units of hexuronic acid and N-acetylglucosamine. The saccharide units can be sulfated at various positions and epimerization may also occur along the chain. These modifications influence interactions of the HS chain with effector proteins such as cytokines and chemokines. Determining the ratio of these different structures is important in understanding the mechanisms underlying several diseases. Analysis of intact HS chains is practically impossible by instrumental analytical tools due to their large size (up to 70 kDa). Characterization of the average sulfation pattern is usually performed after enzymatic hydrolysis of the polymeric chain into the constituent disaccharide units. However, HPLC-MS analysis of HS disaccharides poses a challenge from both chromatography and mass spectrometry sides, due to their diverse polarity and unfavorable ionization characteristics.

The aim of our work was to systematically investigate the chromatographic effects of solvent composition, salt concentration, and salt type in isocratic HILIC-WAX separations of HS disaccharides building on our previous results [1]. Acetonitrile-water ratio of the solvent highly influenced both the elution characteristics and ionization efficiency. Altering the salt concentration improved elution characteristics and did not cause ion suppression. Based on these results, we developed a salt gradient operating with self-packed HILIC-WAX μHPLC columns coupled to ESI mass spectrometry working in negative ion mode. Using the salt gradient improved sensitivity and repeatability could be achieved, compared to previous methods using the same resin. It was possible to separate and quantify the unsaturated HS disaccharides down to a few femtomoles, using a relatively short, 20-minute-long gradient.

Application of the described method was demonstrated in case of biological examples. Sulfation patterns of heparan sulfates determined using the present method enabled HS structural characterization from limited sample amount.

Acknowledgements

Supported by the ÚNKP-18-3-II-BME-230 New National Excellence Program of the Ministry of Human Capacities. LT and KV acknowledge the support of the National Research Development and Innovation Office (OTKA PD 121187, and OTKA 119459) and LT is grateful for the support of the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

References

1 Turiák L *et al* J Chromatogr A. 2018;1544:41-48.