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This is to officially certify that

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participated in the

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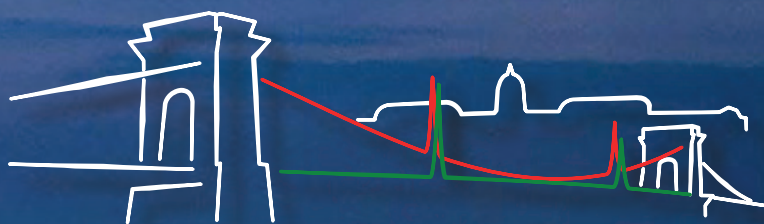
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BOOK OF ABSTRACTS

HILIC and Graphite-based Solid-phase Extraction of Heparan Sulfate Disaccharides

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Heparan sulfate (HS) is a class of glycosaminoglycans built up of disaccharide repeating units comprised of glucuronic/iduronic acid and glucosamine residues; sulfation of the disaccharide building blocks may happen at four different positions. The most common workflow for HS analysis is enzymatic digestion followed by solid-phase extraction (SPE) purification and HPLC-MS measurement. Optimization of the SPE purification of HS disaccharides from biological matrices is an important, but usually neglected part of the analysis.

We aimed to develop SPE methods for maximizing the recovery of the HS disaccharides during sample preparation. First, we developed a HILIC purification method based on self-packed cotton pipet tips. The sample loading, elution conditions, and solvent volumes were thoroughly optimized. We found that the cotton-HILIC method provided excellent (ca. 80%) recovery for the highly hydrophilic doubly and triply sulfated disaccharides, but sample loss up to 60% was observed during the sample loading step for the less hydrophilic ones. Second, we tested three different commercial graphite SPE systems with three different loading and elution protocols, respectively. Excellent recovery was observed for the less hydrophilic non-sulfated and monosulfated components with most of the methods, while the highly-sulfated ones were not eluted from the column.

As a result, we designed a combined method where the flow-through of the cotton-HILIC SPE is further purified with the graphite-based system. The method was applied for biological matrices like HS chain isolated from different species, and we concluded that it was suitable for bias-free analysis of small amounts of biological samples.

Keywords: heparan sulfate, glycosaminoglycan, purification, solid-phase extraction, HILIC