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Online Electrokinetic Sample Cleanup and Evaluation Method for APTS Labeled N-Glycan Separation by Capillary Electrophoresis

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Summary

Capillary electrophoresis (CE) is one of the most frequently used liquid phase separation techniques for the analysis of complex carbohydrates. Since glycans in most of the cases lack chromophore or fluorophore groups, their CE analysis usually requires tagging by a charged fluorophore. To speed up the derivatization reaction, a large excess of the labeling reagent is typically used, therefore, a purification step is necessary prior to CE analysis using the industry standard low pH gel-buffer system. In addition to representing an extra sample preparation step with the associated labor and cost, the purification process also holds the risk of losing some of the sample components. In this presentation we demonstrate an online electrokinetic sample cleanup process with electroosmotic flow (EOF) assisted separation in a bare fused silica capillary using alkaline pH background electrolyte and normal polarity of separation voltage [1]. 8-Aminopyrene-1,3,6-trisulfonic acid (APTS) labeled maltooligosaccharides were analyzed first to understand the complex effect of the downstream EOF and the counter current electromigration of the sample components including the labeling dye. The use of 150 mM caproic acid - 253 mM Tris (pH 8.1) running buffer facilitated the entrance of the sample components of interest into the separation capillary, while the excess labeling reagent was excluded, therefore, did not interfere with the detection. The alkaline caproic acid - Tris running buffer was then applied to the N-glycome analysis of human serum samples, showing excellent separation performance, and more importantly, without the need of the extra sample purification step. Next to the radically new separation method, the required novel data evaluation method will be introduced as well [2].

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References

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