



CERTIFICATE OF PRESENTATION

This is to certify that

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presented

Poster presentation: “Combined solid phase extraction purification method for heparan sulfate analysis”

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Prof Frédéric Lynen, Ghent University, Belgium
Chair, HTC 17th Symposium

Combined solid phase extraction purification method for heparan sulfate analysis



INTRODUCTION

Glycosaminoglycans (GAGs) are long linear-chained polysaccharide molecules built up of repeating disaccharide units. These macromolecules are often connected to a protein core as post translational modification, exerting immense biological roles. **Heparan sulfate (HS)** building units consist of a hexuronic acid connected to an *N*-acetylglucosamine, which can contain sulfation in different positions.

The analysis of these disaccharide building blocks is a widespread techniques for HS investigation. **Before the HPLC-MS measurements, a purification step is necessary, in order to remove the matrix and residual digestion components. However, due to the wide polarity range of these disaccharides, measurement and purification are still a challenging task.**

AIMS

Development of solid-phase extraction (SPE) method for small amount of HS disaccharides

- Self-packed cotton-HILIC SPE method
- Commercial graphite based SPE methods



CONCLUSIONS

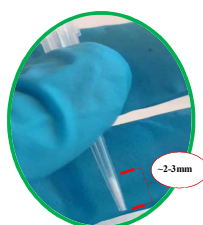
- Cotton-HILIC: for more polar components
- Graphite: for non polar components
- Combined method: ultimate for all HS disaccharides
- Application on biological samples is feasible



Self-packed cotton-HILIC method

Commercial 100% cotton pads.

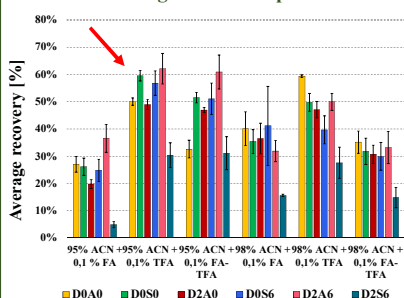
HILIC-type resin
Loaded into pipette tips into 2-3 mm height



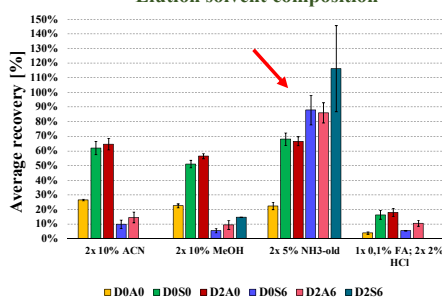
Optimized parameters:

- cotton amount
- composition of loading
- acid type
- acid concentration
- composition of elution
- temperature
- solution volumes

Loading solvent composition



Elution solvent composition



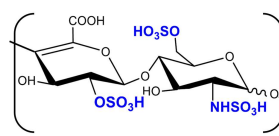
Optimized protocol

Centrifugation parameters: 2500 rpm, 1 min

1. Activation with 50 µl 40% H₂O 60% ACN
2. Conditioning with 50 µl 1% TFA, 95% ACN x 2
3. Loading with 30 µl 1% TFA, 95% ACN
4. Reload x 2
5. Washing with 50 µl 1% TFA, 95% ACN
6. 40°C heated elution with 10 µl 5% NH₃ 2x

Lawrence-code

Hex-GlcNAc	D0A0
Hex-GlcNS	D0S0
HexA2S-GlcNSNAc	D2A0
HexA-GlcNSNAc6S	D0A6
HexA-GlcNS6S	D0S6
HexA2S-GlcNS	D2S0
HexA2S-GlcNAc6S	D2A6
HexA2S-GlcNS6S	D2S6



Graphite-based methods

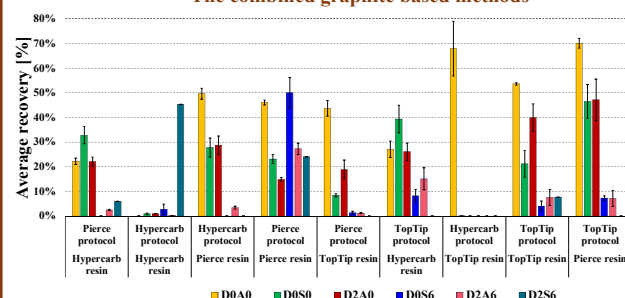
Three type commercial graphite base solid phase extraction resins.



Three protocols by the manufacturers

All possible combinations

The combined graphite based methods



Optimized protocol

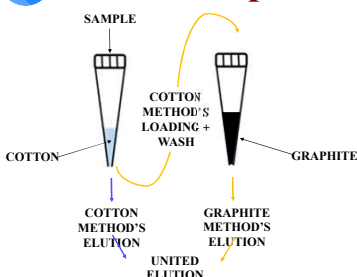
Centrifugation parameters: 2500 rpm, 1 min

1. Activation with 100 µl 80 % ACN 0,1% TFA 2x
2. Conditioning with 100 µl water 2x
3. Loading in 50 µl water. Incubation 2 min before centrifuging.
4. Reload 1x
5. Washing with 100 µl water 3x
6. Elution with 50 µl 40% ACN 0,05% TFA 3x

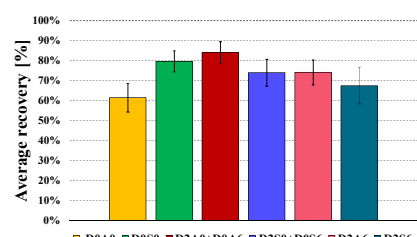
Better applicable for the less polar, none- and monosulfated disaccharides



Combined protocol

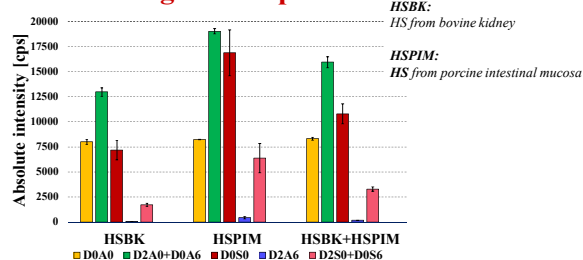


Standard mixture



✓ RECOVERY > 60% ✓ NO SIGNIFICANT BIAS IN SULFATION PATTERN

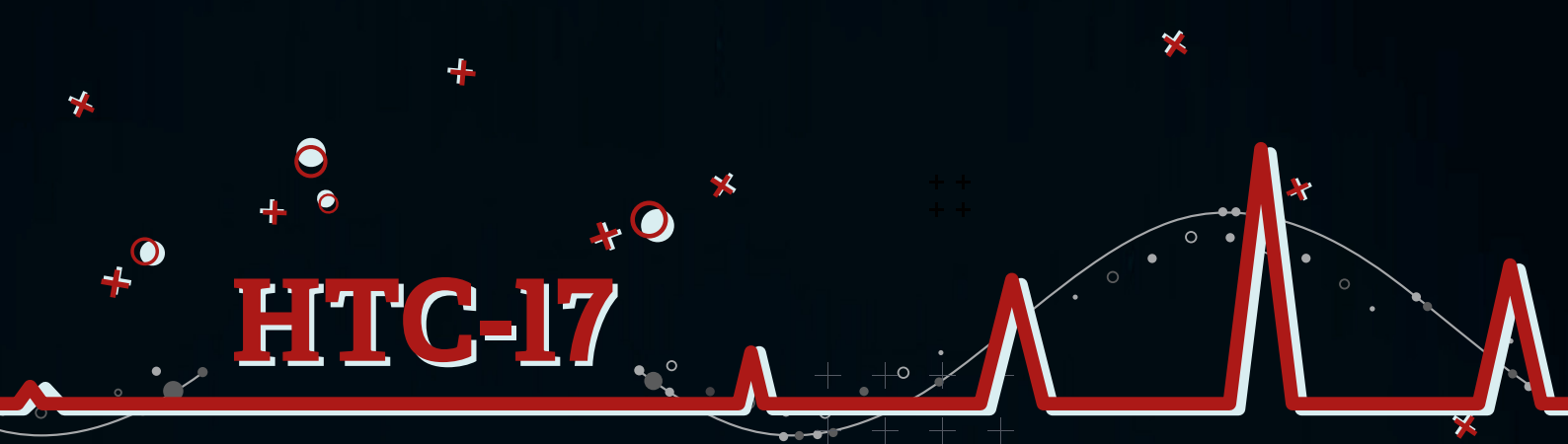
Biological samples



✓ WELL APPLICABLE

Acknowledgments

National Research, Development and Innovation Office (OTKA PD 121187 and OTKA FK 131603) and János Bolyai Research Scholarship of the Hungarian Academy of Sciences.



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Combined solid phase extraction purification method for heparan sulfate analysis

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Glycosaminoglycans (GAGs) are long-chained macromolecules that are built up of sugar disaccharide repeating units. These molecules are often attached to proteins as post- translational modifications, thus having an important role in many biological processes. Heparan sulfate is one of the subclasses of GAGs that has the most variable sulfation features of all. The repeating disaccharide units can be sulfated at several positions and can contain up to three sulfate groups. This structural variability gives a wide range of polarity to them and makes HPLC separation quite complicated. After enzymatic digestion, a purification step is necessary to remove contaminants and salts from the sample. However, due to the diverse physicochemical properties of these disaccharides, developing an all-fit method is still a challenging task.

We aimed to develop a solid phase extraction (SPE) method, that could be used for purification of HS disaccharides extracted from small size biological samples.

In the first phase of the project, we developed a method operating with self-packed cotton SPE spin tips (HILIC-type resin). We tested the effect of several parameters on the recovery of HS disaccharides: the brand and amount of cotton used, quality and concentration of acids used for loading, pH and salt content of the elution. The operation temperature and solvent volumes were also optimized. According to our results, the developed system binds well the more polar, more sulfated components, while a significant loss was observed for the non-sulfated and singly-sulfated components during sample loading.

In the second phase, we tested graphite-based commercial SPE systems for GAG purifications. During our experiments, we combined and applied the manufacturers recommended protocols to all three type of solid phases, in order to test the effect of these protocols on different resins. Our experiments showed that graphite-based resins are ideal for purification of non-sulfated and singly sulfated components, but the more sulfated ones are bound too strongly to the solid phase.

As a result, we found that the two optimized methods complement each other, therefore to achieve high recovery and undistorted sulfation pattern, a combination of the two methods is necessary. The flow-through of the cotton SPE is further purified on the graphite phase and the two elution fractions are combined.

Finally, to test the applicability of the combined method on small-size biological samples, we purified the digested samples of heparan sulfate chains originating from bovine kidney (HSBK) and porcine intestinal mucosa (HSPIM). Results showed significant improvements as compared to previously used methods.

Keywords: solid phase extraction, sample purification, glycomics, glycosaminoglycan, HILIC