



CERTIFICATE OF ATTENDANCE

This is to officially certify that

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Research Centre for Natural Sciences

from

Hungary

participated in the

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Attila Varga

ISC2022 Symposium Secretariat

Cotton-HILIC and Graphite Based Solid Phase Extraction Purification Methods for Chondroitin Sulfate Disaccharides

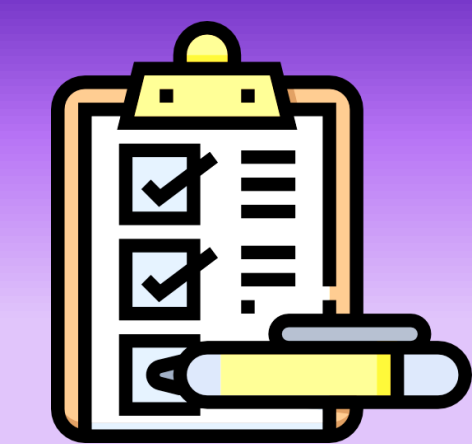


INTRODUCTION

Glycosaminoglycans (GAGs) are long linear 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. Chondroitin sulfate (CS) building units consist of N-acetylgalactosamine (GalNAc) and glucuronic acid, and they can contain sulfation in different positions.

The disaccharide building block analysis is a widespread technique for CS investigation. Before the HPLC-MS measurements, a purification step is necessary to remove the matrix and residual digestion components. However, due to the wide polarity range of these disaccharides, measurement and purification are still challenging tasks.

AIMS



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

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

CONCLUSIONS



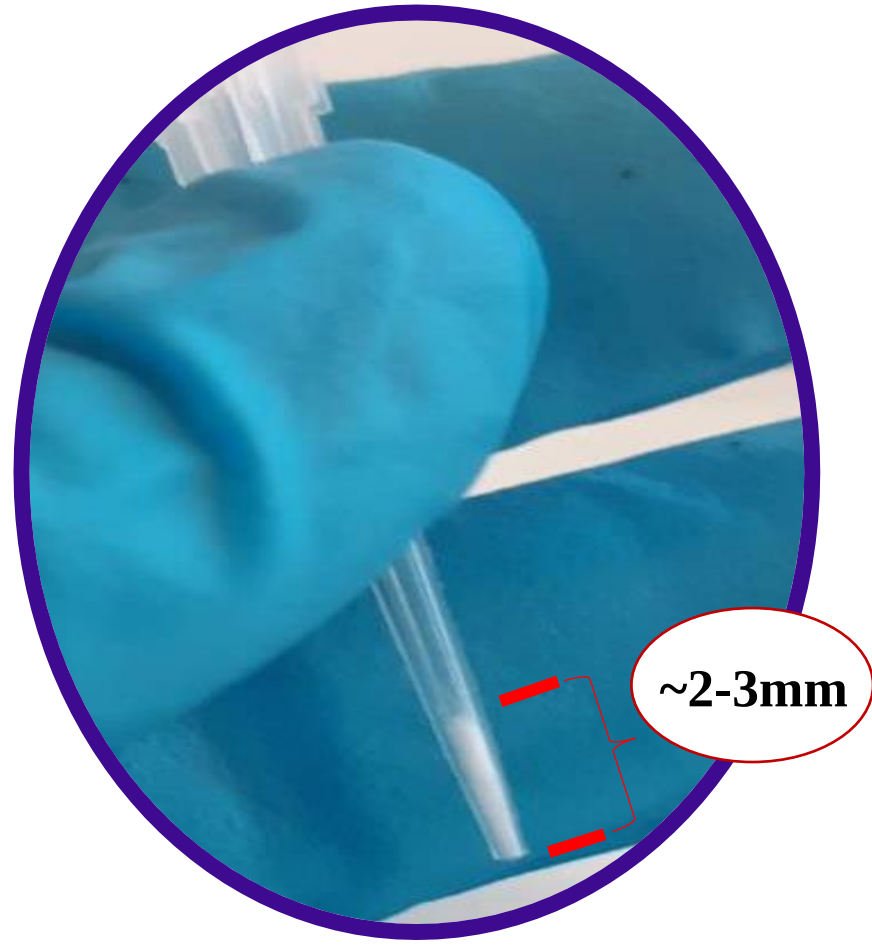
- Cotton-HILIC: for more polar components
- Graphite: for non-polar components
- Combined method: ultimate for all CS disaccharides
- Application on biological samples is feasible

Self-packed cotton-HILIC method

Commercial 100% cotton pads.

HILIC-type resin

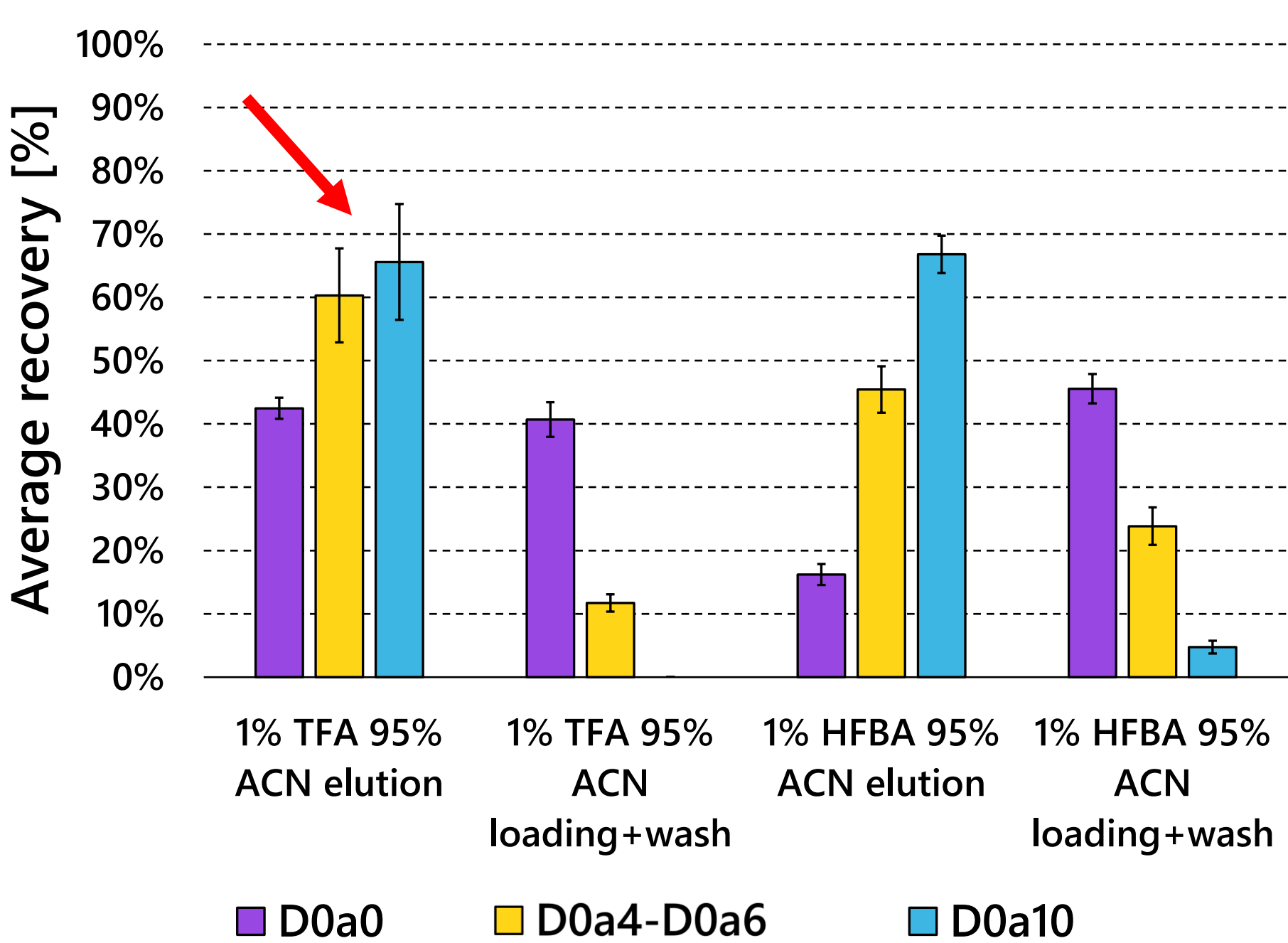
Loaded into pipette tips into 2-3 mm height



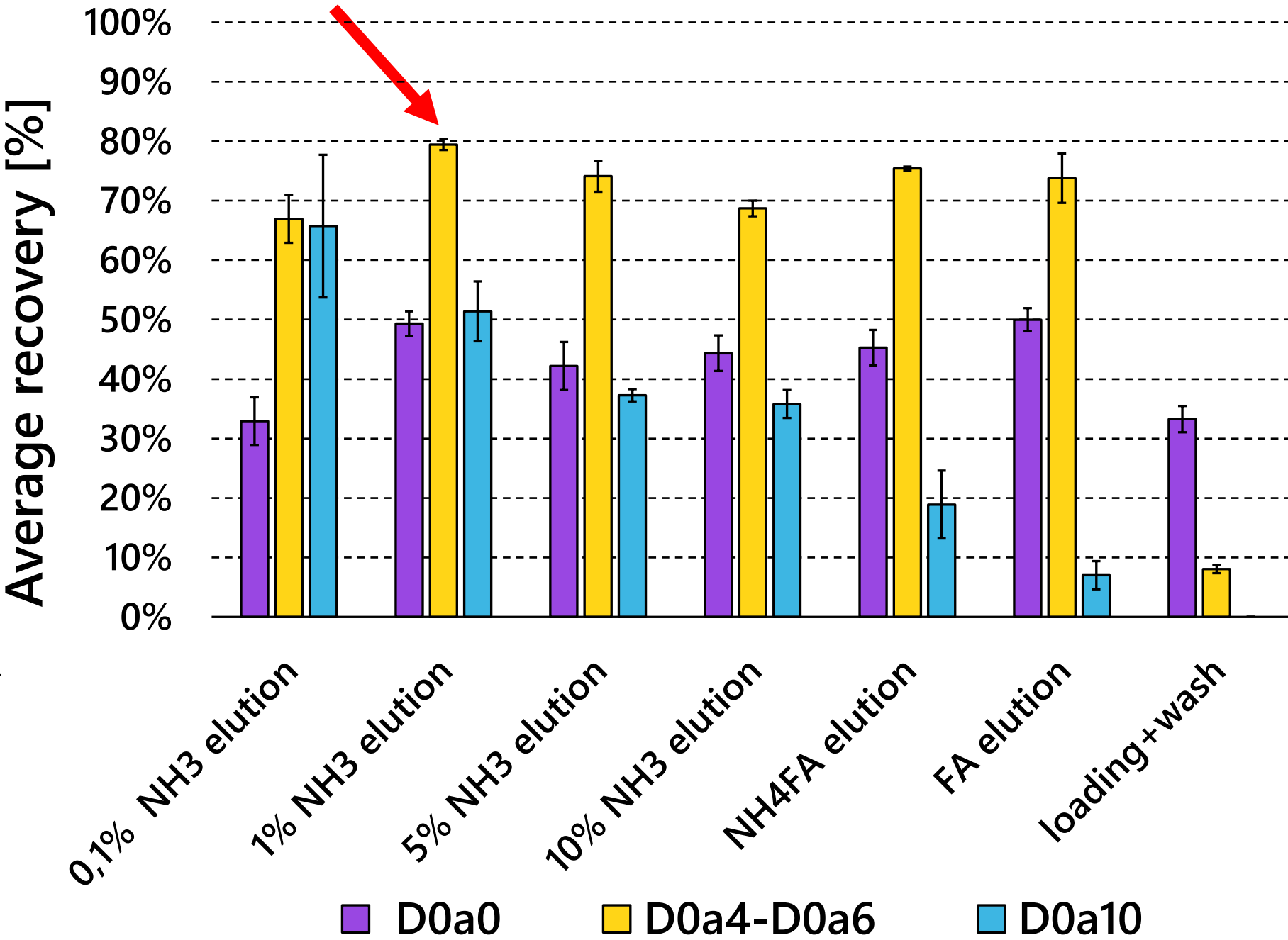
Optimized parameters:

- composition of loading
- acid type
- acid concentration
- composition of elution

Loading solvent composition



Elution solvent composition



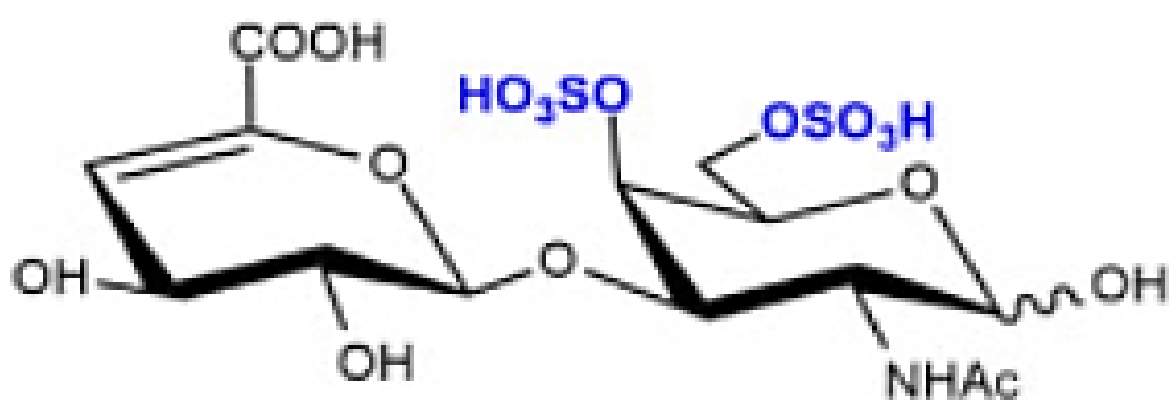
Optimized protocol

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 1% NH₃ 2x

Lawrence-code

ΔHexA-GalNAc	D0a0
ΔHexA-GalNAc4S	D0a4
ΔHexA-GalNAc6S	D0a6
ΔHexA-GalNAc4S6S	D0a10



Better applicable for the more polar disaccharides

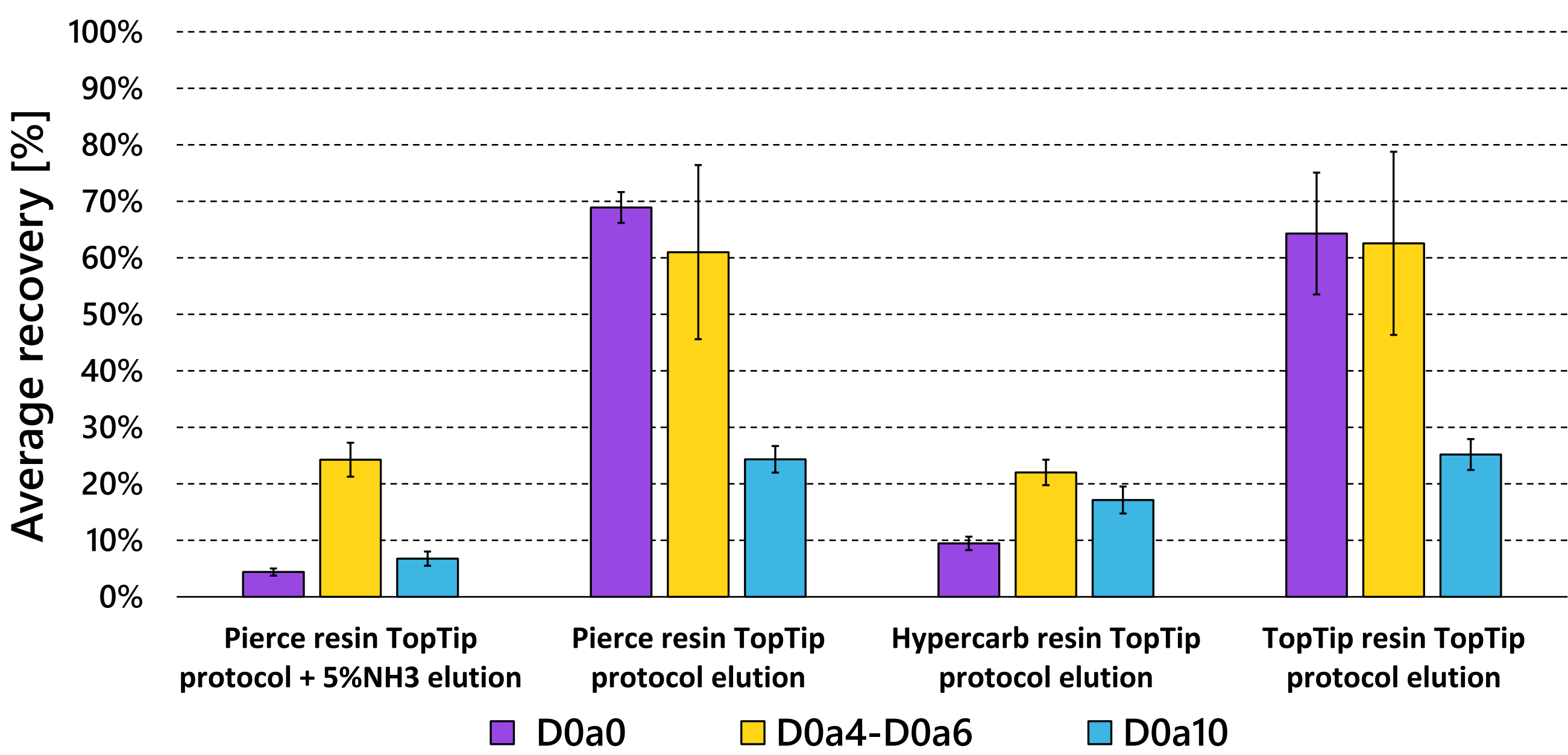
Graphite-based methods

Three commercial graphite-based solid-phase extraction resins



TopTip protocol by the manufacturer

Graphite-based methods



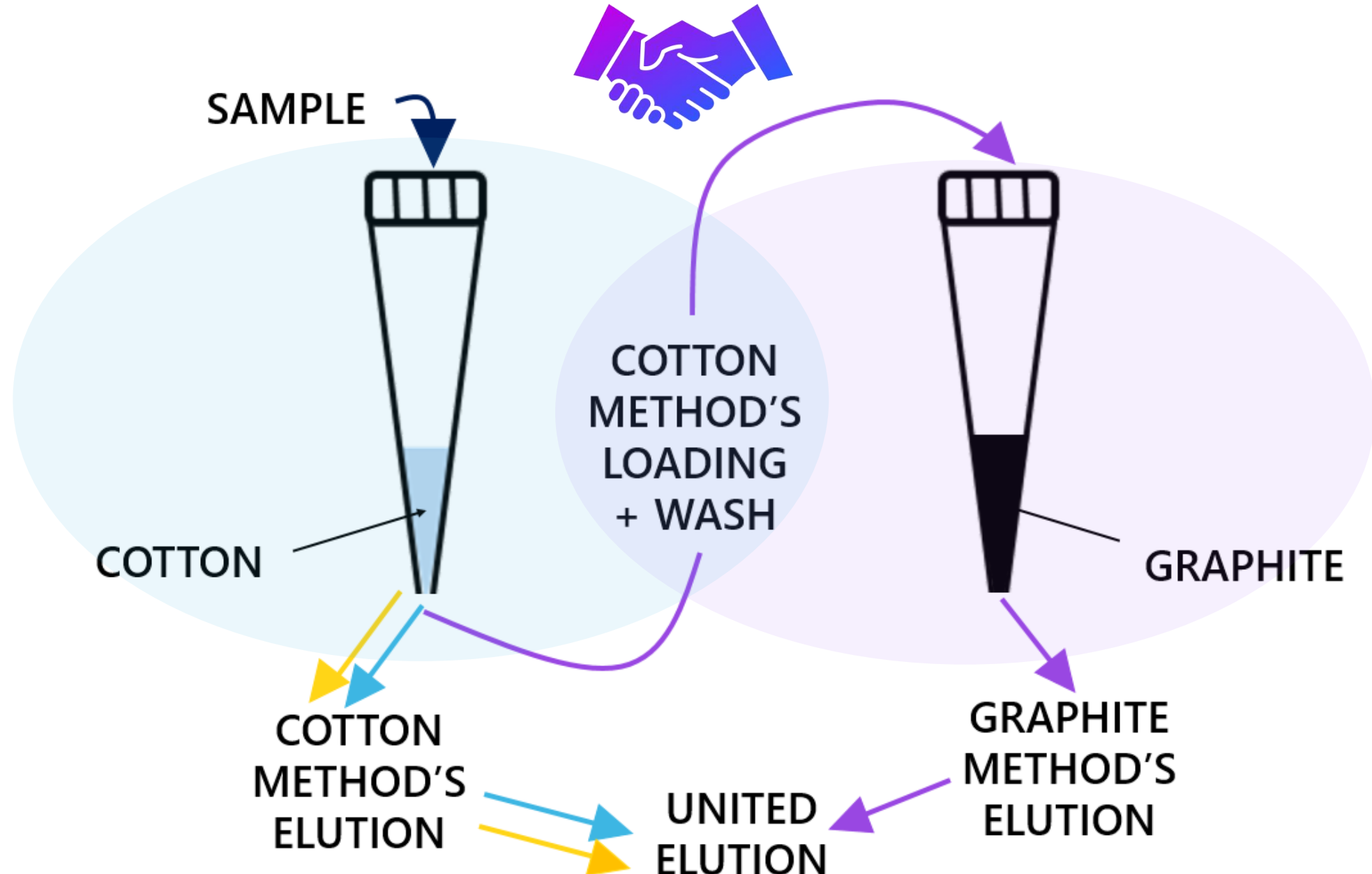
Optimized protocol

Centrifugation parameters: 2500 rpm, 1 min

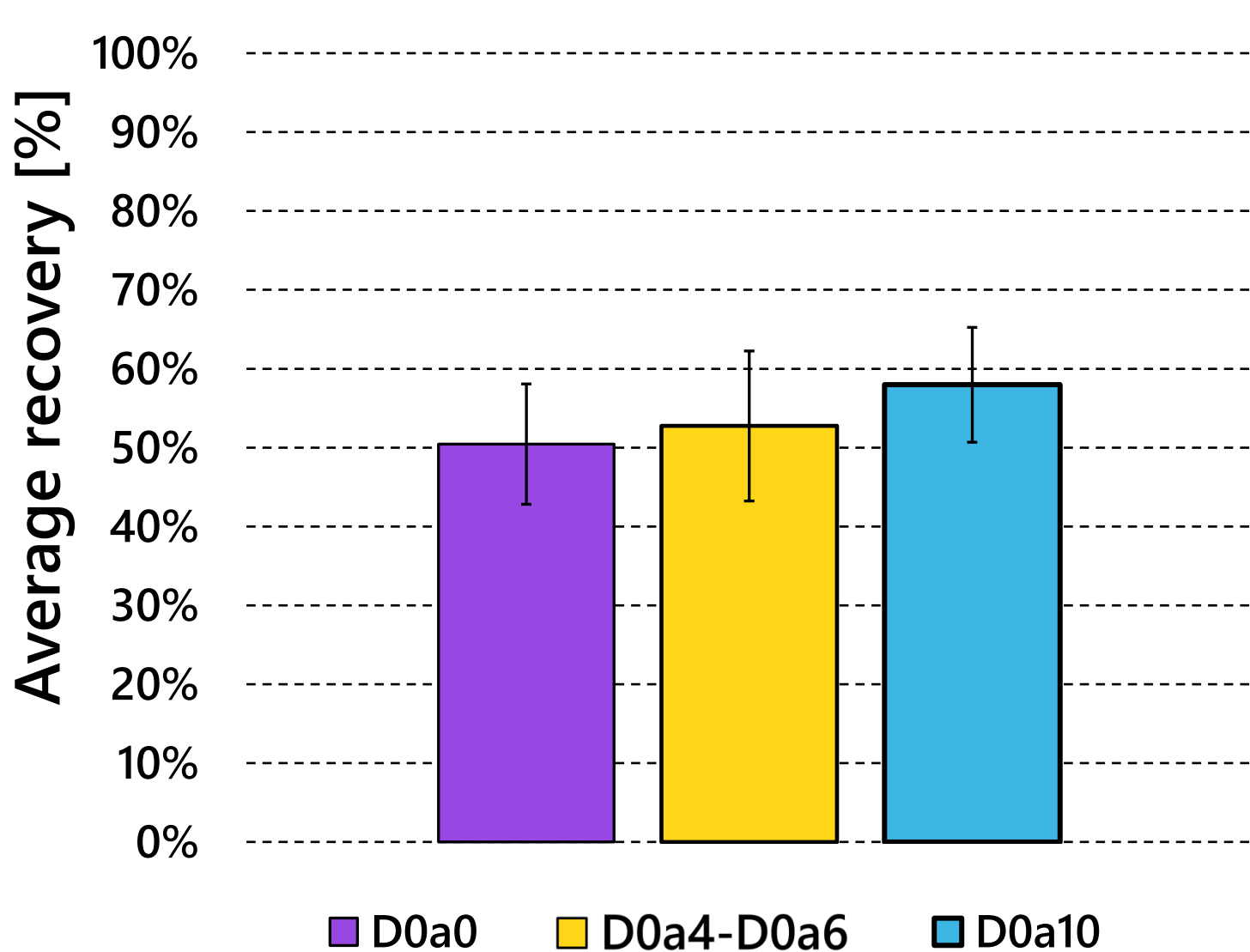
1. Activation with 100 µl 80 % ACN 0,1% TFA 2x
2. Conditioning with 100 µl water 2x
3. Loading in 50 µl water. Incubation 2 min before centrifuging.
4. Reload 1x
5. Washing with 100 µl water 3x
6. Elution with 50 µl 40% ACN 0,05% TFA 3x

Better applicable for the less polar disaccharides

COMBINED PROTOCOL

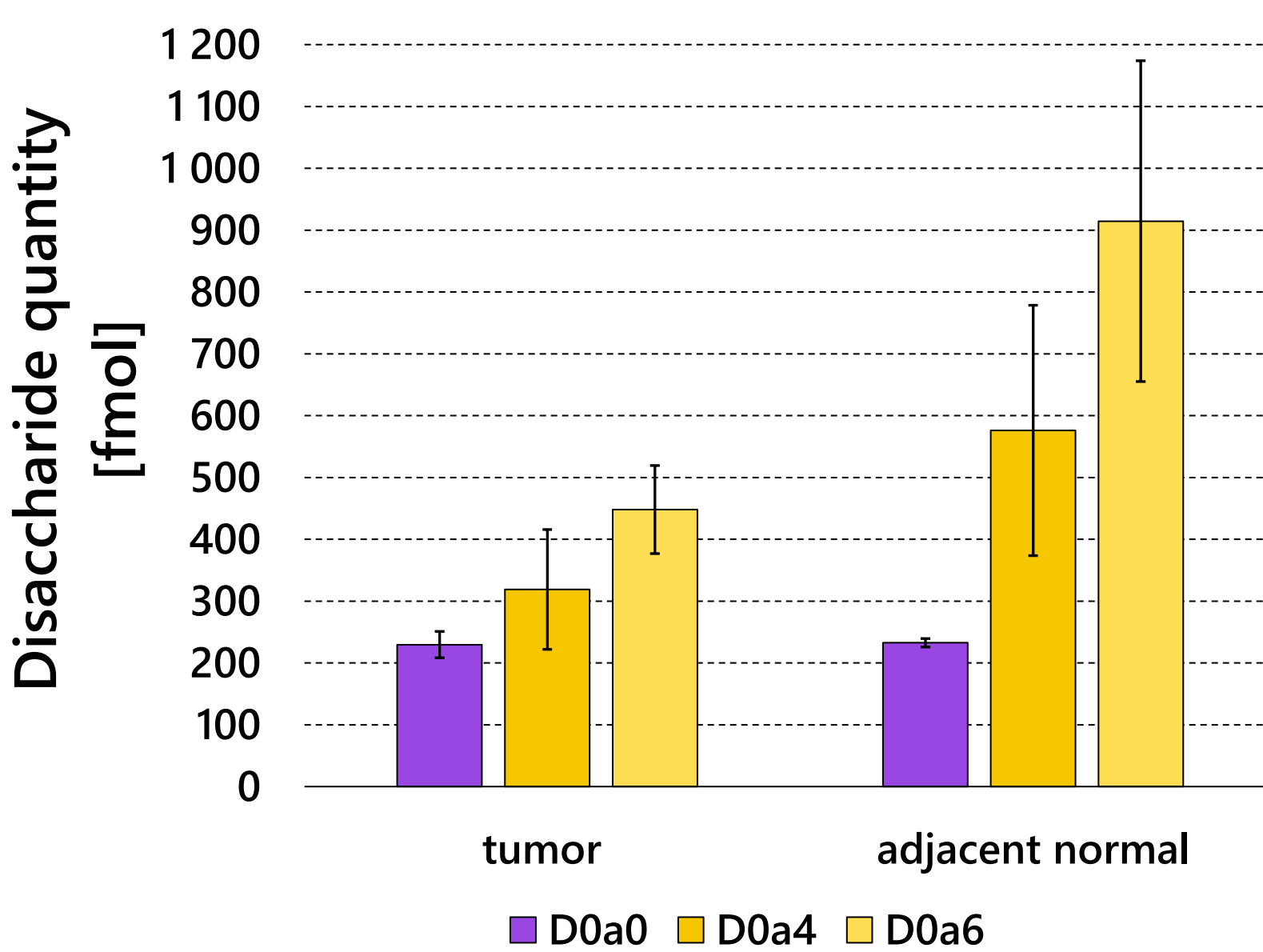


Standard mixture



RECOVERY ~ 50%
NO SIGNIFICANT BIAS IN SULFATION PATTERN

Prostate cancer samples



WELL APPLICABLE

Acknowledgments

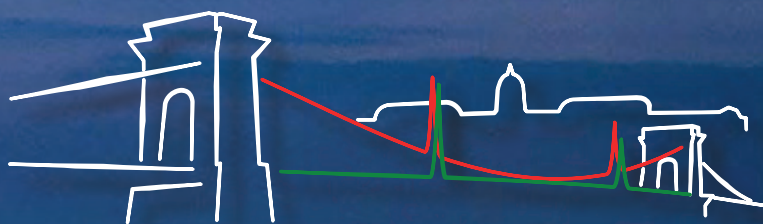
National Research, Development and Innovation Office (OTKA PD 121187 and OTKA FK 131603) and János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

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BOOK OF ABSTRACTS

Cotton-HILIC and Graphite Based Solid Phase Extraction Purification Methods for Chondroitin Sulfate Disaccharides

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Chondroitin sulfates (CS) are long-chained macromolecules built up of repeating disaccharide units. Disaccharide building blocks are frequently analyzed using HPLC-MS following enzymatic digestion of the polysaccharides. Between the enzymatic digestion and the instrumental analysis, a purification step is also necessary to remove contaminants and salts from the sample. We aimed to improve our currently used tissue surface enzymatic digestion method to increase sample extraction of CS disaccharides by testing the digestion efficiency as a function of enzyme amount and solution composition. Another goal was to develop a solid phase extraction (SPE) method, that could be used for purification of CS disaccharides extracted from small size biological samples. We compared different graphite-based commercial SPE systems and tested self-packed cotton SPE spin tips (HILIC-type resin) as well. Using double amount of enzyme, and an extraction solution containing 1% ammonia, successfully improved extraction efficiency. Furthermore, we developed an SPE purification method combining the cotton-HILIC and graphite based methods, and maximized the recovery of CS disaccharides. As the last step we tested the applicability of the methods by investigating prostate cancer tissues.

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