Multivariate Analysis of Tandem Mass Spectrometry Data Distinguishes Epimeric Glycosaminoglycans

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🔖 Introduction

- Glycosaminoglycans (GAGs) are linear polyanionic oligosaccharides composed of consecutive uronic acid and amino sugar residues.
- Chondroitin sulfate(CS) and Dermatan sulfate(DS) have 5-C uronic acid stereochemistry difference.
- Tandem mass spectrometry previously demonstrated for analysis of highly sulfated glycosaminoglycan (GAG); CID, EDD, NETD etc.
- Principal component analysis (PCA) is one of the most favorite techniques in chemometrics to examine the fragmentation patterns of GAGs.
- CID, EDD, and NETD results are compared to identify diastereomers of CS and DS.
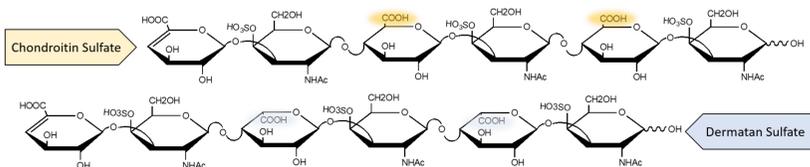
Figure 1. General structural of GAG samples that used, insisting structural difference between glucuronic acid (yellow highlighted) and iduronic acid (blue highlighted).

🧪 Experimental

- CS and DS samples were extracted from natural sources, subjected to enzymatic digestion for this work.

  - **Sample Injection**: 100 uL/h in 50:50 MeOH: H_2O using ESI (conc. 0.2 mg/mL).
  - **CID and NETD** were performed on a 12 T Bruker SolariX.
  - **EDD** was performed on a 9.4 T Bruker SolariX.

- MATLAB software, PLS Toolbox and Metaboanalyst were utilized for a multivariate statistical analysis.

- Blue triangles mean variables (fragment ions) spreading out after the appropriate pre-processing.
- Red diamonds insist samples.
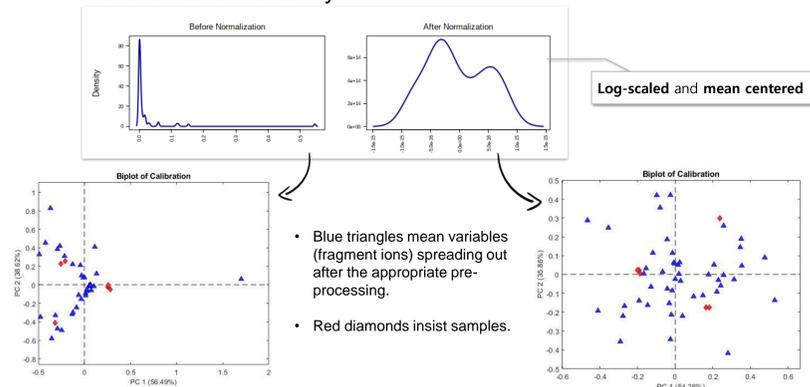
Figure 2. The process of choosing normalization methods.

🔍 Identification Chondroitin Sulfate (CS) and Dermatan Sulfate (DS) using Principal Component Analysis (PCA)
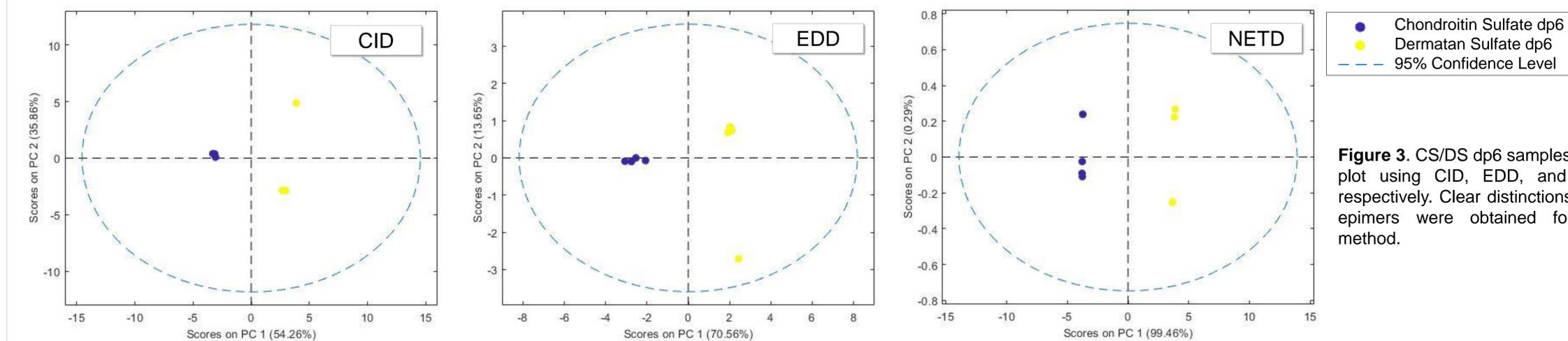
Figure 3. CS/DS dp6 samples scores plot using CID, EDD, and NETD respectively. Clear distinctions of two epimers were obtained for each method.

🔍 Correlation of Three MS/MS methods using PCA
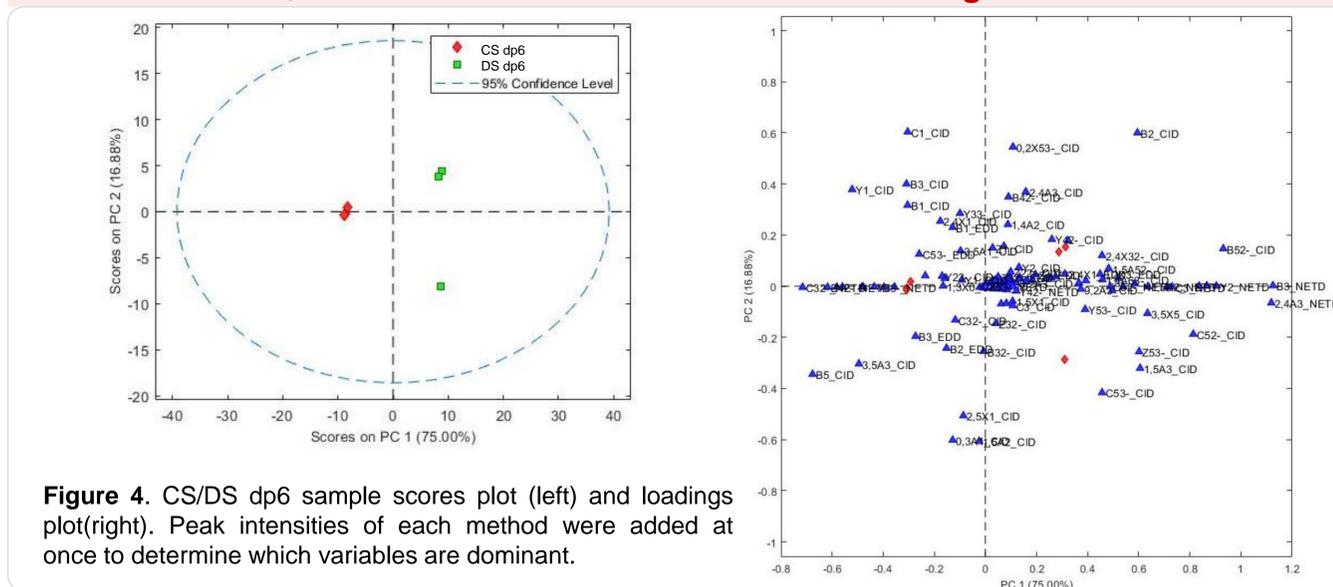
Figure 4. CS/DS dp6 sample scores plot (left) and loadings plot(right). Peak intensities of each method were added at once to determine which variables are dominant.

🔍 Partial Least Squares Discriminant Analysis (PLS-DA)
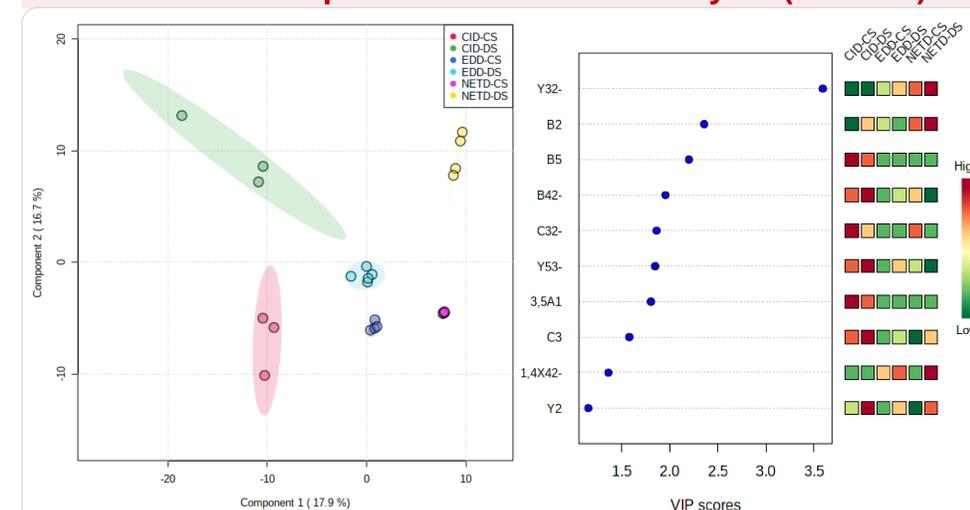
Figure 5. Correlation of three MS/MS methods for CS/DS dp6 samples; Scores plot (left) and its VIP scores plot (right)

✅ Conclusions and Future Directions

- Hexamers of chondroitin and dermatan sulfates can be distinguished by CID, EDD, and NETD.
- For PCA and PLS-DA analysis, log scaling for pre-processing of data produces a better spread in the results, particularly when all three activation methods are processed together.
- Ten dominant ions were suggested by PLS-DA and better discrimination between CS and DS was found in their NETD data.

- A multivariate statistical analysis to highly sulfated/longer CS and DS chains samples will be further implemented to determine structural differences.
- We are working to extend this capability to longer chain GAGs with several classification methods.

♥ References and Acknowledgements

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