

## **Reviewer's Dissertation Thesis Report**

<b>University of Pardubice</b>	
<b>Faculty of Chemical Technology</b>	
<b>Doctoral Study Program</b>	Analytical Chemistry
<b>Student</b>	Mgr. Karel Hořejší
<b>Dissertation Thesis Title</b>	Comprehensive analysis of glycosphingolipids in biological samples using HPLC/MS
<b>Reviewer</b>	PharmDr. Zdeněk Spáčil, Ph.D.
<b>Reviewer's Workplace</b>	Masaryk University, Faculty of Science/Thermo Fisher Scientific Brno

The thesis of Karel Hořejší focuses on the mass spectrometry analysis of glycosphingolipids in biological matrices. Specifically, the author presents a comprehensive theoretical part presenting the structure, classification, and analysis of glycosphingolipids in textbook quality.

The experimental part is limited to a summary of four published papers, two with the author's experimental contribution. The presented extraction protocols and methodology significantly improved the analysis of glycosphingolipids in pancreatic tissue and human plasma. The data on GSL structures are helpful for further research in the field.

The extensive thesis is written thoroughly and clearly. The text is formatted adequately without noticeable mistakes or typos.

I have several comments/questions about the thesis:

- **What was the rationale for selecting deproteinating solvents in study 3.1? Would it be beneficial to test a solvent mixture or another solvent, e.g., isopropanol?**
- **Did you compare the performance of HILIC and RP separation of glycosphingolipids? What are the main pros and cons?**

Masaryk University, Faculty of Science

Kotlářská 267/2, 611 37 Brno, Czech Republic  
E: veda@sci.muni.cz, www.sci.muni.cz


Bank account: KB Brno, Ref No. 85636621/0100, ID 00216224, Tax ID: CZ00216224

- Are the glycosphingolipids in human plasma characterized in study 3.1 as part of an electronic resource (lipid library)?
- I would argue that MS intensity profiling may not directly translate into the GSL abundance in human plasma. Why is that? Did you attempt to mitigate that?
- Would study 3.2 benefit from the use of HRMS, and how?

### Conclusion

The overall quality of the presented thesis is high; it fulfills all requirements, and I highly recommend this thesis for defense.

Date 29.10.2024



.....  
Signature

Masaryk University, Faculty of Science

Kotlářská 2, 602 00 Brno, Czech Republic  
E: veda@sci.muni.cz, www.sci.muni.cz

Bank account: KB Brno, Ref. No.: 85636621/0100, ID: 00216224, Tax ID: CZ00216224