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University of South Bohemia Faculty of Science

České Budějovice, June 16, 2018

Ref.: **Biological Chemistry BSc 2018 Defence, June 18th, 2018, 14:15-15:00**

Bachelor Thesis Assessment: Felix Leibetseder

Tutor:	RNDr. Petr Šimek, CSc.
Opponent:	Ing. Pavla Fojtíková Ph.D.
Committee Chairman:	Prof. RNDr. Libor Grubhoffer, CSc.
Committee Members:	Ing. David Kahoun, Ph.D. Tatyana Prudnikova, MSc. Ph.D. Dmitry Loginov, MSc. Ph.D.

The Thesis Title: **The Biochemistry of Bile Acids and Their HPLC-MS Analysis.**

Felix Leibetseder started his Bc. study and laboratory experiments at the Laboratory of Analytical Biochemistry & Metabolomics of the Biology Centre on the research of bile acids before two years. Bile acids represent complex, often conjugated chemical structures and an important part of the human metabolome that have not been investigated in the University Campus yet. The main goal of the bachelor study was therefore to find experimental conditions enabling comprehensive bile acid analysis in human urine and serum/plasma.

Felix successfully reviewed current biochemical knowledge and current analytical methods applied to their analysis. Throughout the study, he acquired basic skills essential for the unassisted operation of the current HPLC-MS technology, in particular, reversed-phase liquid chromatography coupled to electrospray triple quadrupole mass spectrometry. In the experimental part, he investigated analytical properties of 14 bile acids and later also 4 reference deuterated bile acids. In the study, electrospray ionization ion source conditions, multiple reaction monitoring (MRM) conditions and fast HPLC separation were optimized for the comprehensive bile acid analysis. Calibration of bile acids in neat solvents and depleted serum matrix were tested and the metabolite set measured in urine a serum plasma. The obtained data indicate that (i) higher sample volumes and more sensitive, latest generation MS instrument must be used for the intended



comprehensive analysis. The reported results thus serve as a valuable outcome for future complete method validation and its application with the cooperating JKU university group in metabolomics research.

Although Felix has heavily been engaged in two study programs of the Austrian and Czech syllabus, his approach has still been enthusiastic and was capable to accomplish the biochemical and analytical challenges encountered during the work-up of the bachelor study. Our regular communication and numerous discussions proceeded smoothly so I concluded that Felix is a skilled, active student capable to acquire the bachelor level and participate successfully on the future master programs and relevant projects.

Considering the personal qualities and capability of Felix to adapt to the demanding university studying conditions, I would like to appreciate his approach and the results accomplished. Therefore I highly recommend the Thesis to be defended before the University Committee.

Petr Šimek