Table 5.2 Course specification

Type and level of studies: Bachelor Academic Studies
Course name: HPLC in Biochemistry
Course status: elective
Number of ECTS credits:6
Requirement: none
Course aim To provide students with systematic knowledge in high-performance liquid chromatography, with focus on application inbiochemistry and related fields. To provide students with practical skills in development and application of HPLC methodsin solving biochemical and related problems.
Course outcome After completing the course, student is able to: (1) describe the fields of use of liquid chromatography in modernbiochemical, medicinal and related investigations, (2) demonstrate knowledge of HPLC hardware and parameters, andtheir effects on analysis results, (3) independently select, adapt and develop new chromatographic methods for solvingbiochemical and related problems, (4) perform HPLC and MS-specific laboratory procedures and techniques, (5) performdata analysis and critically evaluate results of HPLC-DAD and HPLC-MS analysis.
Course content *Theory* Basic concepts in chromatography, and performance parameters. Retention mechanisms in HPLC, HPLC modes,optimization of chromatographic separation. HPLC hardware (pumps, injectors, columns, detectors) – principles, limitations, effects of settings on analysis results. Qualitative HPLC analysis – identification by retention, basics ofUV/VIS, API-MS and NMR spectrometry. Quantitative LC analysis – data processing, optimization of quantitativeHPLC-UV/VIS, HPLC-MS and HPLC-FLD methods, validation. Basics of preparative HPLC. Sample preparation forHPLC analysis. HPLC analysis of primary and secondary biomolecules (amino acids, peptides and proteins,carbohydrates, lipids, vitamins, nucleic acids and their monomers, plant phenols, terpenoids and alkaloids) – samplepreparation, chromatography, detection, spectral characteristics, identification. Application of HPLC in biochemistry andrelated fields – pharmaceuticals, food, forensics, clinical diagnostics, biochemical investigations. *Practice:* Familiarization with HPLC-DAD instrument and software. Preparation and qualitative HPLC-DAD analysis of selectedplant material. Development of quantitative HPLC-DAD method for selected natural products. Software for HPLCseparation optimization. Familiarization with HPLC-MS-MS instrument and software. Demonstration of MSn techniques,interpretation of ESI-MS spectra. Development of quantitative HPLC-MS/MS method for selected natural products.Interpretation of ESI-MS spectra of proteins (molecular weight determination), peptides (*de novo* sequencing) andtriacylglycerols (structure elucidation).
Literature 1. Weston A, Brown PR (1997): HPLC and CE – principles and practice, Academic Press, San Diego, USA 2. Lough WJ, Wainer IW (1995): High performance liquid chromatography – Fundamental Principles and Practice,Blackie Academic & Professional, London, UK

Number of classes of active teaching				Other classes
Lectures: 3 (45)	Practice: 2 (30)	OFT:	SRW:	

Teaching methods
Lectures, laboratory work, consulting, e-learning (OERs), seminar

Assessment of knowledge (maximum of 100 points)			
Pre-exam obligations	**Points**	**Final exam**	**points**
activity during lecture classes	**5**	written exam	70
practical teaching	**10**	oral exam	
seminars	**15**		