Course Unit Descriptor

Study Programme: Bachelor Academic Studies in Biochemistry
Course Unit Title: HPLC in Biochemistry
Course Unit Code: IB-405
Name of Lecturer(s): Associate professor Dejan Orčić
Type and Level of Studies: Bachelor of Science Degree
Course Status (compulsory/elective): elective
Semester (winter/summer): winter
Language of instruction: English
Mode of course unit delivery (face-to-face/distance learning): Face-to-face
Number of ECTS Allocated: 6
Prerequisites: none
Course Aims: To provide students with systematic knowledge in high-performance liquid chromatography, with focus on application in biochemistry and related fields. To provide students with practical skills in development and application of HPLC methods in solving biochemical and related problems.
Learning Outcomes: After completing the course, student is able to: (1) describe the fields of use of liquid chromatography in modern biochemical, medicinal and related investigations, (2) demonstrate knowledge of HPLC hardware and parameters, and their effects on analysis results, (3) independently select, adapt and develop new chromatographic methods for solving biochemical and related problems, (4) perform HPLC and MS-specific laboratory procedures and techniques, (5) perform data analysis and critically evaluate results of HPLC-DAD and HPLC-MS analysis.
Syllabus: *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 of UV/VIS, API-MS and NMR spectrometry. Quantitative LC analysis – data processing, optimization of quantitative HPLC-UV/VIS, HPLC-MS and HPLC-FLD methods, validation. Basics of preparative HPLC. Sample preparation for HPLC 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) – sample preparation, chromatography, detection, spectral characteristics, identification. Application of HPLC in biochemistry and related fields – pharmaceuticals, food, forensics, clinical diagnostics, biochemical investigations. *Practice* Familiarization with HPLC-DAD instrument and software. Preparation and qualitative HPLC-DAD analysis of selected plant material. Development of quantitative HPLC-DAD method for selected natural products. Software for HPLC separation 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) and triacylglycerols (structure elucidation).

Required Reading:

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

Weekly Contact Hours:	**Lectures: 3**	**Practical work: 2**

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

Knowledge Assessment (maximum of 100 points): 100

Pre-exam obligations	points	**Final exam**	points
Active class participation	5	written exam	70
Practical work	10		
Seminar	15		