Level: bachelor

Course title: Liquid Chromatographic Methods in Biochemistry (IB-405)

Status: elective

ECTS: 6

Requirements: none

Learning objectives

To provide students with advanced knowledge in high-performance liquid chromatography, with focus on application in biochemistry, medicine and phytopharmacy. 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, biomedicinal and phytochemical investigations, (2) demonstrate advanced 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

Theoretical instruction

Basic concepts in chromatography. Retention mechanisms in LC, LC modes, optimization of chromatographic performance. LC detectors – principles, limitations. Qualitative LC analysis – hyphenated techniques, basics of UV/VIS and API-MS spectrometry. Quantitative LC analysis. LC methods validation. Preparation of biological and other samples for LC analysis. LC-UV/VIS and LC-MS analysis of secondary (phenolics, alkaloids, terpenoids, N- and S-compounds) and primary biomolecules (lipids, carbohydrates, amino acids and proteins, nucleic acids, vitamins, hormones) – isolation, UV/VIS spectral characteristics, fragmentation mechanisms. Chemometrics in chromatography, chemotaxonomy. Metabolomics, biomarkers. Application in enzyme kinetics study, protein-ligand interaction study, detection of disease markers, drug analysis, narcotics analysis etc.

Practical instruction

Mastering the HPLC-DAD and HPLC-DAD-MS-MS instruments and software. Developments and optimization of method for quantitative HPLC-DAD determination of selected natural products. Demonstration of MSⁿ techniques, interpretation of API-MS spectra. Preparation and qualitative HPLC-DAD-MS-MS analysis of selected plant material or herbal drug. Developments and optimization of method for quantitative HPLC-MS-MS determination of the selected metabolites in material of animal or human origin.

Weekly teaching load				Other:
Lectures: 3	Exercises: 3	Other forms of teaching:	Student research:	