Subject: Advanced Analytical Chemistry for Life Sciences (AACLifeSci)

SYLLABUS

3 ECTS

30 hours of attendance

- Course/Subject: Advanced Analytical Chemistry for Life Sciences (AACLifeSci)
- 2nd Semester
- Postgraduate course
- Modality *ON CAMPUS* course
- Prerequisites: Bachelor in Chemistry or equivalent
- Area of knowledge: Analytical Chemistry

COURSE DESCRIPTION

Metabolomics, proteomics and lipidomics are disciplines related to the other "omics " but all of them have specific requirements, workflows, and are different as far as the type of information they provide is concerned. The course aims to give postgraduate students knowledge that would allow them to identify their potential for providing valuable information in the field of Life Sciences. To achieve this, the course will first cover the main aspects of molecular mass spectrometry, including the most recent developments in instrumental design and their advantages and disadvantages along with understanding of mass spectral processes for structural elucidation. Secondly, it will cover fundamentals of high performance separation techniques coupled to mass spectrometry, separation mechanisms, instrumentation, sample treatment, and applications.

Finally, the course will cover the types of metabolomic, proteomic, and lipidomic studies based on the different approaches. Strategies for data processing will also be described. The different stages of these “omics” analysis, their strengths and weaknesses will also be assessed through a critical study of several previously published articles. Apart from that, some of the bioinformatics resources used nowadays will be described.

1. Mass spectrometry. Lectures 7h

1.1. Introduction & General concepts about MS
1.2. Instrumentation: sources, analysers and detectors
1.3. Tandem MS/MS
1.4. Hyphenated techniques. Couplings.
1.5. Structural elucidation. Fundamentals of fragmentation of organic molecules.

This project has been funded with support from the European Commission.

2. Separation techniques coupled to mass spectrometry. Lectures 3h

2.1. LC-MS
- 2.1.1. Main concepts about LC-MS
- 2.1.2. Separation Mechanisms. Main principles of RP-LC & HILIC. Nano LC.
- 2.1.3. Instrumentation for (U)HPLC
- 2.1.4. Optimization of LC-MS separations

2.2. GC-MS
- 2.2.1. Main concepts about GC-MS. Retention Index
- 2.2.2. Instrumentation of GC-MS. RTL
- 2.2.3. 2D-GC
- 2.2.4. Sample pre-treatment. Derivatization.

2.3. CE-MS
- 2.3.1. General concepts about CE-MS
- 2.3.2. Separation Mechanisms. Main principles of CZE & MEKC
- 2.3.3. Instrumentation for CE-MS

Proteomics + Metabolomics + Lipidomics. Lectures 15h (5h +5h +5h)

METABOLOMICS: 5 h

3.

3.1. Introduction to metabolomics. Main concepts.

3.2. Analytical process in metabolomics

3.3. Experimental Design

3.4. Quality Control and Quality Assurance Procedure in Metabolomics

3.5. Data processing. Reprocessing, alignment, normalization, scaling, filtering. Statistical analysis.

3.6. Identification. Data bases. Confirmation.

3.7. From data identification to pathways

3.8. Biomarker validation

3.9. Clinical and biochemical applications

LIPIDOMICS: 5h

4.

4.1 Introduction to lipidomics

4.2. Analytical approaches in lipidomics
- 4.2.1. Experimental Design
- 4.2.2. Sample preparation
- 4.2.3. Targeted profiling of lipid classes
- 4.2.4. MS-based lipid identification: concepts

4.3. Data processing and identification of lipids
- 4.3.1. Targeted and non-targeted lipidomics
- 4.3.2. Lipidomic bioinformatics tools

4.4. Data analysis
- 4.4.1. From data identification to pathways
- 4.4.2. Functional interpretation of the results

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PROTEOMICS: 5h

5.1. Introduction to Proteomics
- 5.1.1. Detection and quantification of protein levels
- 5.1.2. Detection and quantification of protein modifications
- 5.1.3. Detection and quantification of subcellular protein localization
- 5.1.4. Detection and quantification of protein interactions

5.2. Analytical approaches in proteomics
- 5.2.1. Experimental Design
- 5.2.2. Sample preparation
- 5.2.3. MS-based protein identification: concepts
- 5.2.4. Proteomic approaches for the identification of proteins
- 5.2.5. Technique for protein identification: PMF vs PFF
- 5.2.6. Quantitative proteomics
- 5.2.7. New technologies

5.3. Data processing and Identification of proteins
- 5.3.1. Protein sequence databases
- 5.3.2. Search engines

5.4. Data analysis
- 5.4.1. From protein identification to pathways
- 5.4.2. Functional interpretation of the results

Practical Lessons: 5h

2 approaches: targeted and non-targeted. Sample: mammal cells.

a) Proteomics with free online tools
- i) Generation of FASTA database from Uniprot (SwissProt)
- ii) Transformation of data to mzML with MSConverter in ProteoWizard
- iii) Search Engines: OMSSA and X!Tandem with SearchGUI
- iv) Generation and evaluation of results: PeptideShaker for peptide and protein visualization, and validation. PTM analysis

b) Metabolomics with free online tools.

Data preprocessing for LC-MS & GC-MS

workflow4metabolomics: Analysis of metabolomics data
- i) Preprocessing
- ii) Statistics
- iii) Annotation

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LEARNING OUTCOMES:

After completing this course the student should be able to:

1. Review critically the available types of mass analysers and ionization methods, their advantages and disadvantages including modes of tandem mass analysis.
2. Recognize fragmentation patterns of organic molecules mainly from small metabolites, proteins and lipids.
3. Discuss in a comprehensive way the different mechanisms of separation coupled to MS for metabolomics, proteomics, and lipidomics.
4. Know and understand the different methods of sample treatment and their limitations.
5. Identify all stages of the study and the different approaches involved.
6. Discuss the use of public software in data reprocessing and pathways analysis.
7. Acquire basic skills in the use of useful databases as open access resources.
8. Explain to non-specialists how these three "omics" disciplines can be expected to provide valuable information in different areas of Life Science.

TEACHING AND LEARNING ACTIVITIES:

- Lectures and workshops: 25 hours
- Practical Lessons: 5 hours
- Student centred learning: 60 hours
- Total student effort: 90 hours

ASSESSMENT OF LEARNING:

CLASS ATTENDANCE

- In order to be eligible for examination, students must attend at least 75% of scheduled class time (attendance sheets will be used).
- 100% attendance at practical classes is required.

ASSESSMENT CRITERIA:

- Examination on completion of teaching period: Public oral communication on a related topic and a corresponding report weighting 45% and 45 % respectively
- Practical lessons: 10%

REFERENCES:

1. Gary L. Glish, Richard W. Vachet. The basics of mass spectrometry in the twenty-first century. Nature Reviews Drug Discovery (2003) 2, 140-150.
2. Fred W. McLafferty. A Century of Progress in Molecular Mass Spectrometry. Annual Reviews of Analytical Chemistry (2011) 4, 1–22.

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3. James J Pitt. Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry . Clinical Biochemistry Reviews (2009) 30, 19-34.
4. Annalaura Mastrangelo, Alessia Ferrarini, Fernanda Rey-Stolle, Antonia Garcia, Coral Barbas. From sample treatment to biomarker discovery: A tutorial for untargeted metabolomics based on GC-(EI)-Q-MS. Analytica Chimica Acta. (2015) 900, 21-35.
5. Antonia Garcia, Shama Naz, Coral Barbas. Metabolite fingerprinting by capillary electrophoresis-mass spectrometry. Methods Mol Biol (2014) 1198, 107-223.
6. Shama Naz, Antonia Garcia, Magdalena Rusak, Coral Barbas. Method development and validation for rat serum fingerprinting with CE-MS: application to ventilator-induced-lung-injury study (2013), 405(14) 4849-4858.

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