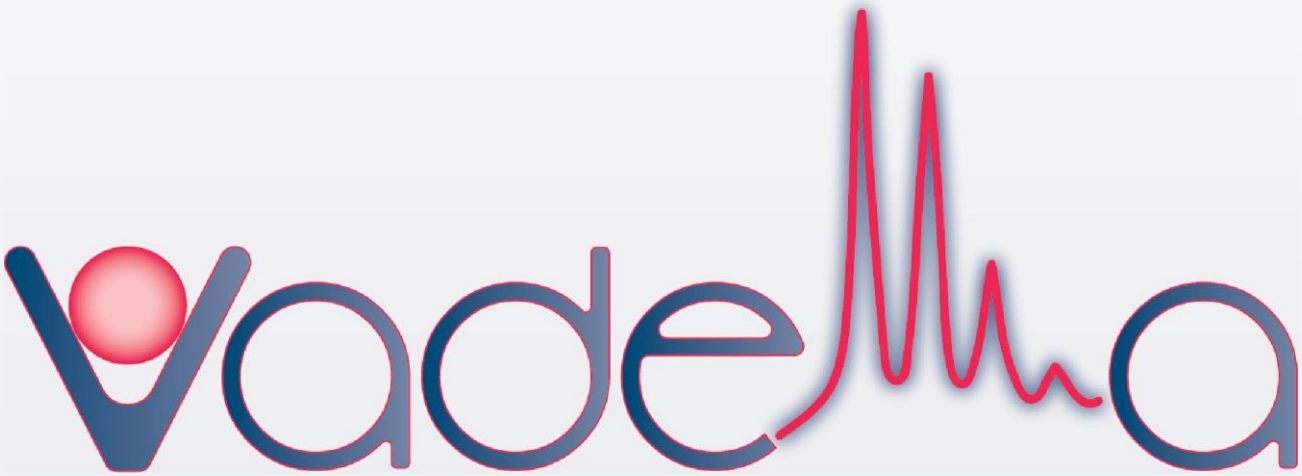

Second Workshop

"Application of Mass Spectrometry-Coupled to Separation Techniques in Bioanalytical Chemistry"

07th - 11th January 2019 - University of Copenhagen - Denmark

Proceedings

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 675879

INTRODUCTION

VADEMA (Doctoral Industrial School for Vaccine Design through Structural Mass Spectrometry) aims at delivering industry-oriented PhD training in the field of structural mass spectrometry applied to vaccinology.

The four recruited Early-Stage Researchers (ESRs) experience an intersectoral training programme encompassing a 18-months internship in Glaxo Smith Kline Vaccines (GSKVACSRL), based in Siena, Italy, and 18-months internship at Department of Pharmacy, in University of Copenhagen (UCPH), DK. The students are enrolled in the Graduate School of Health and Medical Sciences and aThliated to the graduate programme in pharmaceutical sciences, the Drug Research Academy PhD school at UCPH. The 4 Early Stage Researchers (ESRs) are trained in the growing field of Structural Mass Spectrometry and Vaccinology.

For more details and news, visit the website www.vadema.eu

WORKSHOPS

Three workshops will be organized during VADEMA. In these workshops, the ESRs will attend seminars from internationally recognized experts in scientific areas related to the network objectives and participate to practical courses. The topics selected for the workshops aim at covering the state-of-the-art disciplines strongly connected with the VADEMA program.

In particular, the second Workshop in “Structural Mass Spectrometry & Vaccinology” has been organized and hosted by the University of Copenhagen.
The training event entailed with interactive lectures on “Mass Spectrometry Coupled to Separation Techniques in Bioanalytical Chemistry”.
The workshop was conceived as a practical training activity and for this reason the majority of the sessions were performed in laboratory where the fellows performed practical work.

second WorkshoP

Ph.D. course: Mass Spectrometry Coupled to Separation Techniques in Bioanalytical Chemistry

07th -11th January 2019 - University of Copenhagen - Denmark

Day I - Monday 7th January 2019 - Introduction, basic concepts, ionization techniques and mass analyzers

09.00-09.45	**introduction, generally about interpretation of spectra, basic concepts** Christian Skonberg, H. Lundbeck A/S - Denmark
09.50-10.35	**Basic concepts continued** Christian Skonberg, H. Lundbeck A/S - Denmark
10.35-11.00	Coffee break
11.00-11.45	**Basic concepts continued** Christian Skonberg, H. Lundbeck A/S - Denmark
11.45-12.30	Lunch
12.30-13.15	**ionization techniques i: overview of common ionization methods, ei, ci, MALdi** Christian Janfelt, Dept. of Pharmacy, KU - Denmark
13.20-14.05	**ionization techniques ii: Methods used in coupling with Lc and ce. esi (incl. nanospray), APci and APPi** Andreas Kretschmann, Dept. of Pharmacy, KU - Denmark
14.05-14.25	Coffee break
14.25-15.10	**Mass analysers i: Basic concepts, sector field instruments, the quadrupole mass filter and the ion trap** Christian Janfelt, Dept. of Pharmacy, KU - Denmark
15.15-16.00	**Mass analysers i, continued** Christian Janfelt, Dept. of Pharmacy, KU - Denmark

Day II - Tuesday 8th January 2019 - Mass analysers, types of instruments

09.00-09.45	**Mass analysers ii: The FT-icr, the orbitrap and hyphenated instruments** Christian Janfelt, Dept. of Pharmacy, KU - Denmark
09.50-10.35	**Mass analysers iii: ToF and ion mobility spectroscopy** Kasper Dyrberg Rand, Dept. of Pharmacy, KU - Denmark
10.35-1100	Coffee break
11.00-11.45	**ionization techniques iii: icP-Ms** Stefan Stürup, Dept. of Pharmacy, KU - Denmark
11.45-12.30	Lunch
12.30-13.15	**coupled techniques i: gc-Ms** Asger W. Nørgaard, Novo Nordisk A/S - Denmark
13.20-14.05	**The Ms market - commercial instruments** Christian Janfelt, Dept. of Pharmacy, KU - Denmark
14.05-14.25	Coffee break
14.25-15.25	**Mass spectrometry in use – participants' presentations** Christian Janfelt, Dept. of Pharmacy, KU - Denmark
15.30-16.00	**Mass spectrometry in use – participants' presentations** Christian Janfelt, Dept. of Pharmacy, KU - Denmark

second WorkshoP

Ph.D. course: Mass Spectrometry Coupled to Separation Techniques in Bioanalytical Chemistry

07th -11th January 2019 - University of Copenhagen - Denmark

Day III - Wednesday 9th January 2019 - Interpretation of MS spectra and HPLC-MS

09.00-09.45 **interpretation of Ms spectra. exact mass determination, isotopic composition (isotopes and isotope peaks, isotope patterns for larger molecules)**
Line Rørbæk Olsen, H. Lundbeck A/S - Denmark

09.50-10.35 **computer exercise: interpretation of Ms spectra**
Line Rørbæk Olsen, H. Lundbeck A/S - Denmark

10.35-11.00 Coffee break

11.00-11.45 **computer exercise, interpretation of Ms spectra; continued.**
Line Rørbæk Olsen, H. Lundbeck A/S - Denmark

11.45-12.30 Lunch

12.30-13.15 **coupled techniques i: Lc-Ms. hPLc-hardware, columns, separation principles, and method development**
Andreas Kretschmann, Dept. of Pharmacy, KU - Denmark

13.20-14.05 **coupled techniques i: coupling between Lc and Ms**
Andreas Kretschmann, Dept. of Pharmacy, KU - Denmark

14.05-14.25 Coffee break

14.25-15.10 **coupled techniques i: Lc-Ms method optimization**
Andreas Kretschmann, Dept. of Pharmacy, KU - Denmark

15.15-16.00 **coupled techniques i: Lc-Ms method optimization**
Andreas Kretschmann, Dept. of Pharmacy, KU - Denmark

Day IV - Thursday 10th January 2019 - Protein analysis and examples of use

09.00-09.45 **Proteins and peptides, basic concepts**
Kasper Dyrberg Rand, Dept. of Pharmacy, KU - Denmark

09.50-10.35 **Proteins and peptides, examples**
Kasper Dyrberg Rand, Dept. of Pharmacy, KU - Denmark

10.35-11.00 Coffee break

11.00-11.45 **Analysis of protein conformation by Ms: hydrogen-deuterium exchange Ms (hX-Ms)**
Kasper Dyrberg Rand, Dept. of Pharmacy, KU - Denmark

11.45-12.30 Lunch

12.30-13.15 **Mass spectrometry in forensic science**
Petur Weihe Dalsgaard, Dept. of Forensic Medicine, KU - Denmark

13.20-14.05 **Work on Ms problems**
Christian Janfelt, Dept. of Pharmacy, KU - Denmark

14.05-14.25 Coffee break

14.25-15.10 **coupled techniques iii: ce-Ms (p. 228)**
Nickolaj J. Petersen, Dept. of Pharmacy, KU - Denmark

15.15-16.00 **Metabolite identification and characterization in drug development**
Lars Bendahl, H. Lundbeck A/S - Denmark

second WorkshoP

Ph.D. course: Mass Spectrometry Coupled to Separation Techniques in Bioanalytical Chemistry

07th -11th January 2019 - University of Copenhagen - Denmark

DayV - Friday 11th January 2019 - Quantitation and examples of use

09.00-09.45 **Quantitative analysis, basic concepts, isotope labeling, suppression, Limits of detection**
Lars Ynddal, Gubra - Denmark

09.50-10.35 **high throughput screening (hTPs), Pharmacokinetic studies**
Lars Ynddal, Gubra - Denmark

10.35-11.00 Coffee break

11.00-11.45 **Quantitative analysis of biomacromolecules**
Carsten Boye Knudsen, Zealand Pharma A/S - Denmark

11.45-12.30 Lunch

12.30-13.15 **Mass spectrometry imaging**
Christian Janfelt, Dept. of Pharmacy, KU - Denmark

13.20-14.05 **Work on Ms problems**
Christian Janfelt, Dept. of Pharmacy, KU - Denmark

14.05-14.25 Coffee break

14.25-15.10 **Mass spectrometry-based proteomics**
Michael Lund Nielsen, The Novo Nordisk Foundation Center for Protein Research, KU - Denmark

15.15-16.00 **course evaluation and Ms Lab tour at department of Pharmacy, kU**
Christian Janfelt, Dept. of Pharmacy, KU - Denmark

Introduction, generally about interpretation of spectra, basic concept

Christian Skonberg - h. Lundbeck A/s - Denmark

This lecture covered the basic principles of mass spectrometry and how they operate. The lecture was followed by exercises to understand, decipher and create mass spectra along with examples of their applications.

Ionization techniques I: Overview of common ionization methods (EI, CI, MALDI)

Christian Janfelt - dept. of Pharmacy, KU - Denmark

This lecture covered Electron Ionization (EI), Chemical Ionization (CI) and Matrix Assisted Laser Desorption Ionization (MALDI). The lecture was focused on an in-depth explanation of their mechanisms of ionization in order to facilitate a discussion of their respective advantages and disadvantages.

Ionisation methods II

Andreas Kretschmann - dept. of Pharmacy, KU - Denmark

This lecture was focused on the challenges that arise from coupling LC (or HPLC) systems directly to MS systems. Students will learn of the importance of being able to ionization at atmospheric pressure to achieve eThcient coupling between HPLC and MS. Three widespread techniques for this were elucidated: The Electrospray Ionization (ESI), Atmospheric Pressure Chemical Ionization (APCI) and Atmospheric Pressure Photoionization (APPI). The mechanism behind each technique was explained in depth and a discussion of their respective advantages and disadvantages was held.

Mass analyzers I: Basic concepts, Sector field instruments, the quadrupole mass filter and the ion trap

Christian Janfelt - dept. of Pharmacy, KU - Denmark

This lecture describes how we actually measure masses in the mass spectrometer, by giving a detailed description of how sector field, quadrupole and ion trap mass analyzers work. Discussion of advantages and disadvantages of the different mass analyzers, and how they can be applied successfully, will be held.

Massanalysers II: The FT-ICR, the Orbitrap and hyphenated instruments

Christian Janfelt - dept. of Pharmacy, KU - Denmark

This lecture was focused on mass analysers, especially the Fourier Transform Ion Cyclotron Resonance (FT-ICR) and the Orbitrap. There was a brief introduction to hyphenated instruments (combined instruments) e.g. the single quadrupole, the Q-TOF, the Q-Trap, the LTQ-FT Ultra, Q-Exactive, and the Orbitrap Fusion.

Mass analysers III: TOF and Ion mobility spectroscopy

Kasper Dyrberg Rand - dept. of Pharmacy, KU

This lecture also focused on mass analysers with a main focus on the Time Of Flight (TOF) with an introduction to Ion mobility mass spectrometry. For the TOF analyser both history, basic principles, applications as well as pros and cons were presented.

Ionization techniques III: ICP-MS

Stefan Stürup - dept. of Pharmacy, KU - Denmark

This lecture was focused on Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The basics of ICP-MS were presented including instrumentation, the risk of interferences in the sample spectra and sample preparation. There was an introduction to different hyphenated techniques e.g. ICP-MS combined with LC, CE-ICP-MS, SEC-ICP-MS, and GC-ICP-MS.

Coupled techniques I: GC-MS

Asger W. nørgaard - novo nordisk A/s - denmark

This lecture was focused on Gas Chromatography Mass Spectrometry (GC-MS) with information about instrumentation, basic principles, different sample injection strategies and separation te- chniques. In the end there was an introduction to GCxGC (2-dimensional gas chromatography).

The MS market-commercial instruments

Christian Janfelt - dept. of Pharmacy, KU - Denmark

This lecture was a short introduction to the various mass spectrometers on the market and the various vendors. The lecture contained advice on to choose the right mass spectrometer.

Mass spectrometry in use - participants' presentations

Christian Janfelt - dept. of Pharmacy, KU

In the end of the day, the participants discussed mass spectrometry in use by presenting their area of application. Here is a list of some of the applications of mass spectrometry that were presen- ted:

- Quantification of drug compounds
- Identification and characterization of biomarkers
- Analysis of blood samples related to forensic medicine
- Identify metabolites produced after taking specific drugs
- Identification of compounds in plant extract
- Analysis of psychiatric drugs in post-mortem hair
- Identification of peptides in wound-fluids (healing and non-healing)
- Analysis of narcotics, fungi and antibiotics from urine and blood
- Quantification of compounds in complex samples
- AP-MALDI mass spectrometry imaging (MSI) of grain cell wall samples

Interpretation of MS spectra. Exact mass determination, isotopic composition (isotopes and isotope peaks, isotope patterns for larger molecules)

Line Rørbæk Olsen - h. Lundbeck A/s - Denmark

This lecture was an introduction to using ESI-MS for bioanalysis with examples of "real life" applica- tions e.g. metabolic soft spot identification, tentative metabolite identification, screening for reactive metabolites and characterization of therapeutic proteins. There was also introduction to fragmen- tation reactions, isotope pattern of individual elements, and charge states of small molecules and proteins.

Computer exercise: Interpretation of MS spectra

Line Rørbæk Olsen - h. Lundbeck A/s - Denmark

In this lecture, the participants solved various exercises related to interpretation of MS spectra using the information and examples showed in the previous lecture. The software ChemDraw was used for assisting in solving the exercises.

Coupled techniques I: LC-MS. HPLC-hardware, columns, separation principles, and method development

Andreas Kretschmann - dept. of Pharmacy, KU - Denmark

This lecture was an introduction to general principles regarding LC-MS e.g HPLC-hardware, columns, separation and method development. The lecture also contained a description of the steps involved in bioanalysis using HPLC-MS.

Coupled techniques I: Coupling between LC and MS

Andreas Kretschmann - dept. of Pharmacy, KU - Denmark

This lecture focused on different HPLC elements (stationary phase, mobile phase, and application) including HPLC resolution. There was an introduction to parameters that affect HPLC resolution; retention (retention factor k), selectivity (separation factor α) and eThciency (eThciency N).

Coupled techniques I: LC-MS method optimization

Andreas Kretschmann - dept. of Pharmacy, KU - Denmark

This lecture focused on optimization of LC-MS method. For method development, analyte properties, sample matrix and method goals should be evaluated for selection of HPLC mode/conditions, sample preparation and detector selection. MS-instrument optimization, adduct formation and HPLC method development, was also discussed.

Proteins and peptides, basic concepts

Kasper Dyrberg Rand - dept. of Pharmacy, KU - Denmark

This part of the lecture focused on the characteristics of peptides and proteins with respect to

- Physicochemical properties
- Chromatographic properties
- Mass spectrometry analysis

The relevance of appropriate techniques and tools for method development for peptide and protein analysis was elucidated by the following fundamentals:

- Introduction to characteristics of amino acids as building blocks for peptides and proteins, as for optimizing the LC-MS workflow properties such as the pKa values of amino acids need to be taken into account
- Monoisotopic mass and average mass determination based on MS data
- The importance of charge states and isotopic distribution to extract information about mass
- Task: Based on a mass spectrum the monoisotopic mass should be extracted for mass accuracy calculations based on the given formulae

Proteins and peptides, examples

Kasper Dyrberg Rand - dept. of Pharmacy, KU - Denmark

In this section top down and bottom up workflows were compared with regards to their applications based on exemplary cases. Also differences in the application of ESI and MALDI were elucidated. With regards to protein sequencing, a main focus was put on the fragmentation strategy CID and how to extract the according information from MS data, which was demonstrated on exemplary data sets.

Analysis of protein conformation by MS: HX-MS

Kasper Dyrberg Rand - dept. of Pharmacy, KU - Denmark

This session was dedicated to the application of mass spectrometry not only for primary structure, but also for higher order protein structure analysis. The main principles of hydrogen deuterium ex- change mass spectrometry (HDX-MS) were thus covered, including the HDX-MS bottom up workflow , including the following data analysis and interpretation. All aspects were explained with regards to the theoretical background and requirements for a successful HDX-MS experiment.

Mass spectrometry in forensic science

Petur Weihe Dalsgaard - dept. of Forensic Medicine, KU - Denmark

This session gave a general overview over state-of-the-art technical approaches to approach challenges within the field forensic sciences, in which MS is the most used technique. The advantages and disadvantages of different mass spectrometers were pointed out and the challenges of data interpretation were elucidated, including the concepts of targeted and non-targeted screening, as well as semi-quantitative approaches.

Coupled techniques III: CE-MS

Nickolaj J. Petersen - dept. of Pharmacy, KU - Denmark

This session was introducing the advantages of capillary electrophoresis (CE) and CE-MS with a special focus on the little sample volume/amount needed and its consequences for the flow rates applied and a following electrospray ionization. Also various CE-ESI-MS interfaces and recent developments were introduced with according examples for application. Also a brief introduction to microfluidic chips and method optimization was given.

Metabolite identification and characterization in drug development

Lars Bendahl - h. Lundbeck A/s - Denmark

This session gave an introduction to metabolites and biotransformation with regards to metabolites derived from drugs and the according objectives for metabolite characterization. The lecture gave an overview of techniques commonly used for metabolite characterization were explained. Special focus was put on sample preparation and LC-MS/MS with fraction collection and off-line radiochemical detection for sample quantification and characterization. Moreover the lecture gave a short overview over method development for LC-MS-RAD and models for human metabolites were introduced.

Quantitative LC/MS analysis: focus on bio-analysis

Lars Ynddal - Gubra - Denmark

Quantitative analysis refers to the determination of how much of a given component is present in a sample. This lecture was mainly focused on the basic concepts of quantitative analysis, and explaining the main steps for developing and validating a quantitative method for peptides and small molecules in human fluid matrices (as plasma and urine). In regard of the development of the LC-MS method, the HPLC and the mass spectrometers used for quantitative experiments were described and the lecture also gave insights on how to prepare the samples for LC-MS quantitative experiments and how to inspect and minimize ion suppression effect. Concerning the method validation, the concepts of selectivity, accuracy, precision, carry over and how to make a standard curve were presented.

Quantitative LC/MS analysis: bio-analytical applications, HTS-PK studies, small molecules and peptides

Lars Ynddal - Gubra - Denmark

In the first part, the focus was on the importance of the sample preparation for bio-analytical quantitative analysis and the most common methods of clean-up and pretreatments, as protein precipitation, liquid/liquid extraction, solid phase extraction, and on-line turbulent flow chromatography, were reviewed. General aspects of HPLC separation and MS ionization were discussed, together with the optimization of their interphase. In the second part of the lecture, a real example of peptide assay in plasma with high resolution MS was presented, with focus on the internal standard selection and the advantage of the use of multiple internal standards.

Quantitative Bioanalysis of Biomacromolecules

Carsten Boye Knudsen - Zealand Pharma A/s - Denmark

In this lecture an overview of quantitative mass spectrometry (MS)-based bioanalysis of proteins was given. In the first part of the lecture, the advantages and disadvantages of MS-based assays and ligand binding assays were elucidated. A general recap of the theory of ESI-ionization of intact proteins/peptides and their fragmentation was given, which will serve as a support for understanding the principles of LC-MS quantification. In regard of this, the lecture focused on describing the most important parameters for a calibration curve (LOD and LLOQ) and the requirements for selection of internal standards, the principles of reverse phase chromatography, commonly used for the analysis of proteins and peptides, and the most used techniques for protein sample preparation/clean-up. As a conclusion, pro and cons of the analysis of whole protein or analysis of signature peptides after digestion were evaluated, and a real example of protein LC-MS quantitative analysis was showed.

Mass spectrometry imaging of drugs, metabolites and natural products

Christian Janfelt - dept. of Pharmacy, KU - Denmark

When analyzing a plant or animal tissue with a standard LC-MS analysis pipeline, the samples is homogenized and extracted and the extract is analyzed. This allows the identification and also a very accurate quantification of the analytes under investigation; nevertheless, the localization of the compound in the original tissue remains unknown. This limitation was overcame when, in the late 1990, it was demonstrated that MALDI-TOF could be applied to visualize peptides and proteins directly on tissue sections and obtain a spatial information on the localization of the bio-molecules. This technique was called Imaging Mass Spectrometry (IMS). In this lecture different ion source and IMS technique were reviewed, as well with sample preparation procedures. In the last part of the lecture, several applications of IMS were presented: drug absorbance, whole body imaging, biomarker discovery and histology. Also applications on protein imaging were presented, with particular regard on protein imaging of cancerous tissues and application in cancer removal surgery.

Mass spectrometry-based proteomics

Michael Lund Nielsen - The novo nordisk Foundationcenter for Protein research, KU - Denmark

The ability of mass spectrometry to identify and precisely quantify thousands of proteins from complex samples has become an indispensable tool for molecular and cellular biology. This ability extends to the characterization and localization in the protein sequence of post-translational modifications (PTMs), covalent modifications following protein biosynthesis that play a role in protein signaling and interaction, folding or enzymatic activity regulation. Although the cellular 'modificome' is highly complex, only a small subset of the estimated 200 possible types of PTMs is currently amenable for analysis on a global scale. The topic of the lecture was presenting newly developed methodologies enabling proteome-wide characterization of obscure post-translational modifications as SUMOylation, arginine methylation and ADP-rybosilation, based on enrichment of modified peptides after protein digestion and measurement with high-resolution Orbitrap mass analyzer. Given the significant role played by of ADP-rybosilation in disease-related biological processes, the lecture was particularly focused on the developed enrichment method for in vivo characterization of ADP-ribosylation in cultured cells and tissue samples.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 675879