Elisabete Andrade Alves Pires

Degree in Chemistry

Professional Activity Report

Relatório nos Termos do Despacho n.º 20/2010 para Obtenção do Grau de Mestre em Química Bioorgânica, por Licenciados "Pré-Bolonha"

Supervisor: Professor Doutor José Luis Capelo

Júri:

Presidente: Prof. Doutora Paula Cristina de Sério Branco

Vogal: Prof. Doutor José Luis Capelo Martinez

Arguente: Doutor Hugo Miguel Baptista Carreira dos Santos

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

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Relatório de Actividade Profissional

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Abstract

After my graduation in chemistry and specialization in Mass Spectrometry awarded by the Science Faculty of the University of Lisbon in February 2003, I started as a graduated technician at ITQB's Laboratory of Mass Spectrometry in July 2003, having been promoted to senior technician in July 2008. During this time I have been responsible for developing and validating several mass spectrometry and chromatography methods as well as for counseling several proteomics' research groups. I have also been responsible for in-lab quality control and method validation according to ISO 17025 and Good Laboratory Practices (GLP) rules for pharmaceutical industries.

Over 10 years, I gained experienced in the use of different mass spectrometers and HPLC systems from different manufacturers, namely Bruker, Thermo, ABSciex and Waters. I have demonstrated a great capability in its use and deep knowledge about their mode of operation, giving me a high skill for troubleshooting and problem solving.

So far as a result of my research activities, I have published 9 articles in international peer-reviewed journals, two reviews and two book chapters. I have participated in 13 scientific conferences (10 poster presentations (1 best poster award); 6 oral presentations). I have also been involved in the organization committee of 3 scientific workshops.

In addition, I have gained experience in teaching namely, theoretical and practical classes in Mass Spectrometry as part of ITQB´s Ph.D. program and several master classes of different Universities. I have supervised and trained students (Master and Ph.D. students) and scientists (Post-docs) in acquiring skills in sample treatment and mass spectrometry, by implementing new methods (including several laboratory operating procedures and mass spectrometry methods). Furthermore, since April 2013, I have been working as coordinator of mass spectrometry services, being responsible for the method development and research for academics and pharmaceutical industry. Such studies are related to lipidomics, glycomics, metabolomics and phosphoproteomics using mass spectrometry-based approaches.

I also had the opportunity to work for two months in the laboratory of Prof. DePauw in the mass spectrometry laboratory of the University of Liège, Belgium. During this time, I embraced new challenges in the field of mass spectrometry and further improved my leadership skills and professionalism that has always accompanied me throughout my years of work in this field.

My current research interests are the implementation and development of LC-MS-based methodologies and its applications to the study of biological systems, in particular with biomedical and pharmaceutical applications.

Keywords: Chemistry, mass spectrometry, Good Laboratory Practices, pharmaceutical industry, method development and research.

Resumo

Depois da minha graduação em química e estágio em Espectrometria de Massa emitido pela Faculdade de Ciências da Universidade de Lisboa, em Fevereiro de 2003. Comecei como técnico superior de 2ªclasse no Laboratório de Espectrometria de Massa do ITQB em Julho de 2003 tendo sido promovida a técnica superior de 1ª classe em Julho de 2008. Durante esse tempo, fui responsável pelo desenvolvimento e validação de vários métodos de espectrometria de massa e cromatografia, bem como consultora de pesquisa de vários projetos em proteómica.
Fui também responsável pelo controlo de qualidade e validação de métodos de acordo com a ISO 17025 e Boas Práticas de Laboratório (BPL) a pedidos da indústria farmacêutica.

Durante cerca de 10 anos, ganhei experiência na utilização de diferentes espectrómetros de massa e sistemas de HPLC de diferentes fabricantes e modelos, Bruker , Thermo , ABSciex e Waters. Tendo demonstrado uma grande capacidade no seu uso e conhecimento profundo sobre o seu modo de funcionamento, tornei-me responsável pela sua verificação, manutenção e resolução de problemas.

Até agora, como resultado das minhas atividades de investigação, publiquei 9 artigos em revistas e jornais internacionais, dois artigos de revisão e dois capítulos de livros. Participei em 13 conferências científicas (10 apresentações de posters (1 prémio de Melhor Poster); 6 apresentações orais). Estive também envolvida na comissão organizadora de 3 workshops científicos.

Ganhei também experiência em lecionar através de aulas teóricas e práticas em espectrometria de massa, inseridas no programa Ph.D ITQB, e de aulas de mestrado de algumas universidades. Obtive ao longo destes anos experiência na supervisão e orientação de alunos (estudantes de mestrado e doutoramento) e cientistas (pós- docs) como novos utilizadores de espectrometria de massa e na implementação de vários novos métodos. Desde abril de 2013, tenho vindo a trabalhar como coordenadora de serviços de espectrometria de massa e como responsável pela investigação e desenvolvimento de novos métodos para académicos e indústria farmacêutica, com novas abordagens em espectrometria de massa no estudo da lipidómica, glicoproteómica metabolómica e fosfoproteómica.

Tive também a oportunidade de trabalhar durante dois meses no laboratório do Prof. DePauw no Laboratório de espectrometria de massa da Universidade de Liège, na Bélgica e com esta experiência percebi que conseguia abraçar novos desafios, sem colocar em causa as minhas capacidades de liderança e profissionalismo que sempre me acompanharam ao longo dos meus anos de trabalho na área da minha formação académica.

Os meus interesses de pesquisa atuais são a implementação e desenvolvimento de novas metodologias utilizando a técnica de LC-MS e suas aplicações para o estudo de sistemas biológicos, em particular com aplicações biomédicas e farmacêuticas.

Palavras-chave: Química, espectrometria de massa, Boas Práticas de Laboratório, indústria farmacêutica, investigação e desenvolvimento de novos métodos.

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List of abbreviations

APCI- Atmospheric Pressure Chemical Ionization

ER- *Ehrlichia ruminantium*

ESI- Electrospray Soft Ionization

Fig.- Figure

FT-ICR- Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

GIGA- Groupe Interdisciplinaire de Génoproteómique Appliquée

GLP- Good Laboratory Practices

GMP- Good Manufacturing Practices

HPLC- High Pressure Liquid Chromatography

IBET- Instituto de Biologia Experimental e Tecnológica

IGC- Instituto Gulbenkian da Ciência

ITQB- Instituto de Técnologia Química e Biológica

Lab.- Laboratory

LC- Liquid Chromatography

m/z- mass-to-charge ratio

MALDI- Matrix-Assisted Laser Desorption/Ionization

MS- Mass Spectrometry

MS/MS- Fragmentation

MSc- Master of Science

NMR- Nuclear Magnetic Resonance

NVP- Nevirapine

PTM- Post-Translational Modification

QTOF- Quadrupole Time-Of-Flight Mass Spectrometer

RNA- Ribonucleic Acid

RNEM- Rede Nacional de Espectrometria de Massa

S6P– Sucrose-6-Phosphate

SOP- Standard Operating Procedure

SRM- Selected Reaction Monitoring

T6P- Trehalose-6-Phosphate

TiO_2- Titanium dioxide

TOF- Time-Of-Flight

UniMS- Unidade de Espectrometria de Massa do ITQB/IBET

UPLC- Ultra Pressure Liquid Chromatography

1 Introduction

This report aims to obtain the degree of Master of Bioorganic Chemistry, according with the provisions in paragraph 1.b) of Order nº20/2010, this includes graduates over five years of professional experience in the main subject of their degree.

This report presents a detailed account of my professional experience since completion of the degree in chemistry until present, properly substantiated.

2 Personal Information

Complete name: Elisabete Andrade Alves Pires

Nationality: Portuguese

Birth date: 24 November 1973

Phone number: +351 96 636 636 8

Email: epires@itqb.unl.pt

3 Academic degree and Complementary Studies

3.1 Academic Degree

Designation of qualification awarded: Degree in Chemistry, branch of scientific chemistry

Date: 1999-2003

Name and type of the organization providing education or training: Science Faculty of Lisbon University

Thesis stage of degree: "Photodegradation Study of Paraquat Herbicide in aqueous solution by action of TiO2, using ESI-MS/MS"

Thesis final classification: 19 values (19/20)

Degree final classification: 14 values (14/20)

3.2 Complementary Studies

Training of new equipments, software and methods in the field of mass spectrometry and proteomics

Designation of qualification awarded: Training in 2DnanoUPLC / SYNAPT-G2 MSE and Advancing Methodologies for Farm Animal Proteomics.

Date: October 2012 to December 2012

Name and type of the organization providing education or training: Laboratory of Mass Spectrometry, Department of Chemistry, University of Liège, Belgium

Principal subjects / occupational skills: Proteomic analysis of the Rickettsiales *Ehrlichia ruminantium* (ER) bacteria using quantitative proteomics, biotinylation analysis, high resolution mass spectrometry training and opportunity to explore a new software data interpretation, MassLynx.

Designation of qualification awarded: 1st RNEM course on Proteomics

Date: January 2010

Name and type of the organization providing education or training: Instituto de Biologia Experimental e Tecnológica (IBET) and Instituto de Tecnologia Química e Biológica (ITQB) - Universidade Nova de Lisboa, Oeiras, Portugal

Principal subjects / occupational skills: Protein digestion, sample cleaning, sample treatment, Mass spectrometry and Proteomics methodologies and approaches about the different equipments for protein identification and data analysis software tools.

Designation of qualification awarded: 4800 MALDI-TOF/TOF Analyzer and Proteomics Data Analysis

Date: June 2009

Name and type of the organization providing education or training: Applied Biosystems, Darmstadt, Germany

Principal subjects / occupational skills: Exploring data analysis software for proteomics results, sample treatment and possible troubleshooting with the equipment.

Designation of qualification awarded: 2nd Summer Course on Mass Spectrometry in Biotechnology and Medicine.

Date: July 2007

Name and type of the organization providing education or training: Center for Advanced Academic Studies, Dubrovnik, Croatia

Principal subjects / occupational skills: Basics of mass spectrometry applications in biological analysis; theoretical information on glycobiology, protein and peptide fragmentation, sample preparation, imaging and mass spectrometers approaches for biological applications, such as the FT-ICR, Orbitrap, MALDI/TOF-TOF, QTOF, Traps and triple quadrupoles.

Designation of qualification awarded: Practical course "Identification of proteins using Mass Spectrometry Data"

Date: January 2005

Name and type of the organization providing education or training: Mass Spectrometry Laboratory from Instituto de Tecnologia Química e Biológica (ITQB) - Universidade Nova de Lisboa, Oeiras, Portugal

Principal subjects / occupational skills: Further understanding and guidelines about Data analysis software and how to use databases with different algorithms in order to identify proteins from mass spectrometry data

Designation of qualification awarded: Training "Chromatographic Methods Validation"

Date: October 2003

Name and type of the organization providing education or training: Instituto de Biologia Experimental e Tecnológica (IBET), Oeiras, Portugal.

Principal subjects / occupational skills: Linearity, Strength, variances homogeneity, method specificity and evaluation of the system suitability.

Training in GMP (Good Manufacturing Practices) and GLP (Good Laboratory Practices) rules and International standard NP EN ISO 17025

Designation of qualification awarded: Good Manufacturing Practices of pharmaceutical medicines (GMP)

Date: February 2012

Name and type of the organization providing education or training: Instituto de Biologia Experimental e Tecnológica (IBET),Oeiras, Portugal

Principal subjects / occupational skills: National and EU legislation, quality management system in accordance with the regulations, analysis methods validation and documentation.

Designation of qualification awarded: Course Approach to Requirements Management NP EN ISO 17025

Date: June 2009

Name and type of the organization providing education or training: Instituto de Biologia Experimental e Tecnológica (IBET) and Instituto de Tecnologia Química e Biológica (ITQB) - Universidade Nova de Lisboa, Oeiras, Portugal

Principal subjects / occupational skills: quality system, scopes, normative references, terms and definitions, management and technical requirements.

Designation of qualification awarded: Monitoring standardization of owens and fridges.

Date: January 2008

Name and type of the organization providing education or training: Instituto de Biologia Experimental e Tecnológica (IBET) and Instituto de Tecnologia Química e Biológica (ITQB) - Universidade Nova de Lisboa, Oeiras, Portugal

Principal subjects / occupational skills: Maintain efficacy and meet regulatory guidelines.

Designation of qualification awarded: Training on NP EN ISO/IEC 17025:2005 3^{ed}

Date: March 2007

Name and type of the organization providing education or training: Specanalitica, Lisboa, Portugal

Principal subjects / occupational skills: knowledge about quality system, scopes, normative references, terms and definitions, management and technical requirements.

Designation of qualification awarded: Documental management, CAPA, uncertainties and out-of-specification (OOS) in the GLP Unit

Date: February 2007

Name and type of the organization providing education or training: Instituto de Biologia Experimental e Tecnológica (IBET) and Instituto de Tecnologia Química e Biológica (ITQB) - Universidade Nova de Lisboa, Oeiras, Portugal

Principal subjects / occupational skills: Metrological traceability, operational qualification (OQ), OOS results and performance qualification (PQ).

Designation of qualification awarded: GLP methodology information and its application

Date: May 2004

Name and type of the organization providing education or training: Instituto de Biologia Experimental e Tecnológica (IBET), Oeiras, Portugal.

Principal subjects / occupational skills: Methodology, information and its application on the GLP Unit of IBET.

Designation of qualification awarded: Training on verification and calibration of equipment and laboratory material

Date: September 2003

Name and type of the organization providing education or training: Instituto de Biologia Experimental e Tecnológica (IBET), Oeiras, Portugal.

Principal subjects / occupational skills: Micropipettes, balance, glass material and HPLC equipment verification.

Training on uncertainties and statistics analysis applied to chemical laboratories

Designation of qualification awarded: Determination of uncertainties from validation data

Date: January 2008

Name and type of the organization providing education or training: Instituto de Biologia Experimental e Tecnológica (IBET) and Instituto de Tecnologia Química e Biológica (ITQB) - Universidade Nova de Lisboa, Oeiras, Portugal

Principal subjects / occupational skills: Metrological approach to the measurement processes, requiring method validation, establishment of metrological traceability, estimation of measurement uncertainty and monitoring of trends in measurement processes.

Designation of qualification awarded: Uncertainty calculation associated to chemistry analyses

Date: February 2007

Name and type of the organization providing education or training: Instituto de Biologia Experimental e Tecnológica (IBET) and Instituto de Tecnologia Química e Biológica (ITQB) - Universidade Nova de Lisboa, Oeiras, Portugal

Principal subjects / occupational skills: Understanding the uncertainty of the chemical measurements in chemistry analyses.

Designation of qualification awarded: Uncertainty course for chemistry laboratories

Date: March 2006

Name and type of the organization providing education or training: Specanalitica, Lisboa, Portugal

Principal subjects / occupational skills: Concepts of measurements uncertainty analysis and hands-on experience in computing practical examples.

Designation of qualification awarded: Short Statistics course for laboratories

Date: May 2004

Name and type of the organization providing education or training: FORQUAL Human resources and Quality Ltd, Lisbon, Portugal

Principal subjects / occupational skills: Basic concepts and methods of statistics with applications in the experimental biological sciences.

Designation of qualification awarded: Applied statistics to analytical laboratories

Date: December 2003

Name and type of the organization providing education or training: RELACRE, Lisbon, Portugal

Principal subjects / occupational skills: Presentation of the foundations of statistical inference, including the concepts of parameters and estimates and the use of the likelihood function, confidence intervals, and hypothesis tests. Topics including experimental design, linear regression, analysis of two-way tables, sample size and power calculations.

Training on health and safety in laboratory workspace

Designation of qualification awarded: Hands-on course in first aid care, 698/FOR/REC/2007.

Date: October 2007

Name and type of the organization providing education or training: Portuguese Red Cross, Lisboa, Portugal

Principal subjects / occupational skills: Renewal training module designed for first responders and laboratory injuries.

Final classification: 18 values (18/20)

Designation of qualification awarded: Awareness for hygiene and safety at work

Date: February 2007

Name and type of the organization providing education or training: Instituto de Tecnologia Química e Biológica (ITQB) - Universidade Nova de Lisboa, Oeiras, Portugal

Principal subjects / occupational skills: Rules and accomplishment of hygiene and safety at the laboratory.

Designation of qualification awarded: Hands-on course in first aid care, 481/FOR/2005.

Date: November 2005

Name and type of the organization providing education or training: Portuguese Red Cross

Principal subjects / occupational skills: This training module was designed for first responders, health care professional and other workers who are at risk for on-the-job exposure to blood and body fluids that can cause infection. This course helped to meet training requirements for the blood-borne pathogens and laboratory injuries.

Final classification: 18 values (18/20)

Designation of qualification awarded: Training on Health, hygiene and safety at work

Date: June 2004

Name and type of the organization providing education or training: CDRH, Lisbon, Portugal

Principal subjects / occupational skills: Safety orientation, ergonomics, hazard communication, injury & Illness Prevention Plan (IIPP) and Personal Protective Equipment (PPE).

Final Classification: Very good

Others Trainings

Designation of qualification awarded: Short Course on Project Management with Microsoft Project

Date: October 2004

Name and type of the organization providing education or training: CDRH, Lisbon, Portugal

Principal subjects / occupational skills: Knowledge about planning, organizing, and managing resources to complete a specific goal with the desktop tool of Microsoft project software.

Final Classification: Very good

4 Professional Experience

4.1 Resume of Professional Skills

Throughout my professional career I have had hands-on experience in numerous analytical methodologies, namely gel electrophoresis, mass spectrometry-based methodologies, and quality systems for laboratories.

In the area of proteomics I developed and/or implemented mass spectrometry-based methodologies to identification and characterization of proteomes and gel-based methodologies, such as gel electrophoresis, western blotting and in-gel protein digestion.

As responsible for some of the equipments of mass spectrometry at the ITQB´s Mass Spectrometry laboratory I was able to develop and/or implement different methodologies for both academia and pharmaceutical industry. Such methodologies took advantage of the following techniques of analysis: Electrospray Ionization (ESI), Single Reaction Monitoring (SRM), Atmospheric Pressure Chemical Ionization (APCI), microESI, nanoESI, MALDI-TOF/TOF, Synap G2/HDMSE) and Liquid Chromatography (HPLC, UPLC and nanoUPLC) were the main equipments and techniques that I have worked with for the analysis of all kind of compounds, from small molecules to intact proteins, among others. In addition, I was also responsible for the analysis, characterization, quantification and study of the interaction of new drugs in clinical samples by LC-MS and MALDI-TOF/TOF methodologies.

Finally, as the mass spectrometry laboratory was a GLP certified laboratory, I was the person responsible for implementing, verifying and validating the quality under the GLP system according to ISO 17025 and GLP rules for R&D in pharmaceutical industries.

4.2 ITQB/IBET Institutions

Instituto de Tecnologia Química e Biológica (ITQB) is a scientific research and advanced training institute of the Universidade Nova de Lisboa. It is located in the Town of Oeiras, at the Tagus estuary, just outside Lisbon. Since 1996 it has occupied a building inside the campus of the National Agricultural Station, an R&D institution of the Ministry of Agriculture. Some facilities are located in two other buildings, one belonging to the Agricultural Station and the other to the neighboring Gulbenkian Institute. The mission of the ITQB is to carry out scientific research and postgraduate teaching in chemistry, life sciences, and associated technologies, as well as serving the scientific community and to collaborate with the university by promoting activities related to science and technology. The ITQB is provided with excellent research facilities, including equipment and support services. In this context, the Mass Spectrometry Laboratory is included, being responsible for performing the development and validation of analytical methods as well as routine analysis for a broad range of chemical compounds, including small organic and organometalic compounds, peptides, oligosaccharides, nucleotides and proteins.

The Instituto de Biologia Experimental e Tecnológica (IBET) is a private non-for-profit research organization in the area of biotechnology and life sciences. It bridges university and industry research, by establishing partnerships between collaborators, private companies and public institutions, , of which the most relevants are, Instituto de Tecnologia Química e Biológica, ITQB, and the Faculdade de Ciências e Tecnologia (FCT/UNL) in the areas of health-pharma, agro-industry, forestry, agriculture and the environment. IBET is a certified service provider.

In early 2013, the mass spectrometry laboratory service was named as Unit of Mass Spectrometry (UniMS) under a new ITQB/IBET´s management. The goals of the UniMS is to provide state-of-the-art mass spectrometry services to the scientific community and Industry.

4.3 Mass Spectrometry Services

As explained above, the UniMS is the new laboratory of mass spectrometry services operating at ITQB, having replaced the mass spectrometry laboratory services. In this context the earlier equipment existing in the MS lab services are now under the responsibility of UniMS. Services previously provided remain almost the same.

Mass spectrometry is a sensitive technique used to detect, identify and quantitate molecules based on their mass-to-charge (*m/z*) ratio. Originally developed almost 100 years ago to measure elemental atomic weights and the natural abundance of specific isotopes. MS was first used in the biological sciences to trace heavy isotopes through biological systems and later to sequence oligonucleotides and peptides, and to analyze nucleotide structure.

The mass spectrometers available and related HPLCs at UniMS are the following:

- Two Ion Traps mass analyzers, equipped with an ESI, nanoESI and APCI source
- One linear Trap mass analyzer, equipped with an ESI and nanoESI source
- One MALDI-TOF/TOF mass spectrometer
- One HPLC System with UV/Vis detector
- One microHPLC System
- One NanoHPLC System
- One Maldi Spotter

Generally, a typical Mass Spectrometer consists of three parts: ***Ion Source***, ***Mass Analyzer*** and ***Detector*** (Fig.4.1).

An ion source is a device that creates atomic and molecular ions. The function of the ***Mass Analyzer*** is to separate ions with different mass-to-charge (*m/z*) ratios. The Detector records either the charge induced or the current produced when an ion passes by or hits a surface. Finally, the mass spectrum is generated after all data have been collected.

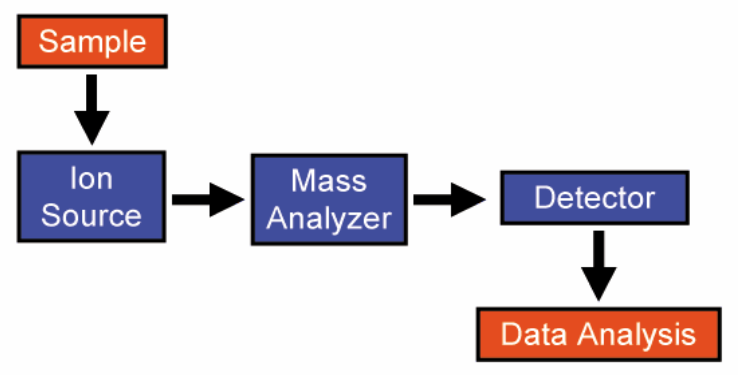


Fig.4.1- Scheme of a Mass Spectrometer
(Source:http://gtms1339.wordpress.com/2013/01/18/363)

The choice of the ionization method depends on the nature of the sample and the type of information asked. So-called 'soft ionization' methods such as electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) tend to produce mass spectra with little or no fragment-ion content.

The term “ionization method” refers to the mechanism of ionization while the ionization source is the mechanical device that allows ionization to occur. The main processes in electrospray ionization are:

A. The sample is dissolved in the solvent, which can be easily evaporated.

B. The solution (mixture of molecules and solvent) is pushed through a very small, charged and usually metal capillary.

C. Due to chemical reactions, the molecules become ionized, surrounded by solvent in small droplets.

D. The droplets are dried so that the ionized compounds are separated from the solvent (Fig.4.2)

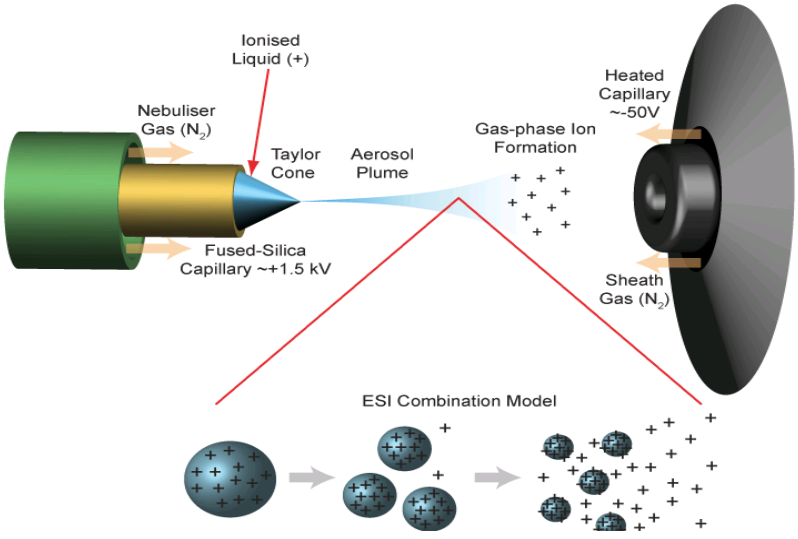


Fig.4.2 - ESI Ionization Process

(Source:www.lamondlab.com/MSResource/LCMS/MassSpectrometry/electrospray)

For MALDI-TOF MS the main processes are as follows:

Samples are prepared by mixing several molecules and embedded in a matrix that will crystallize inside. The matrix is composed of small acid molecules that have a strong optical absorption in the range of the laser wavelength used and play a key role in the vaporization of the analyte. The matrix also serves as a proton donor and receptor, acting to ionize the analyte in both positive and negative ionization modes, respectively. The molecules are then both desorbed and ionized by charge transfer by absorbing the energy of a short laser pulse. Several theories have been developed to explain the ionization from MALDI, one certain is that the ionization depends critically on the matrix–analyte combination, but is not critically dependent on the number of acidic or basic groups of the analyte. This will suggests that a more complex interaction of analyte and matrix, rather than simple acid–base chemistry, is responsible for ionization (Fig.4.3).

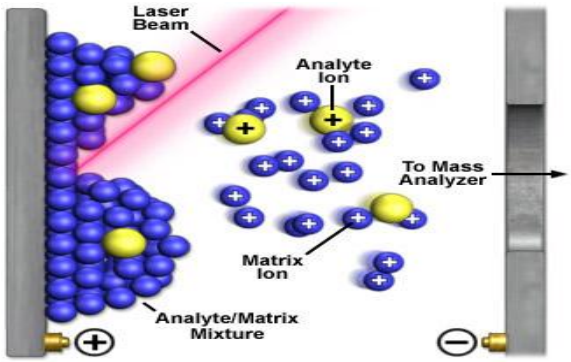


Fig.4.3- MALDI Ionization Process (Source: http://www.siliflow.com)

Similar to ESI ionization is the Atmospheric pressure chemical ionization (APCI), a popular complement to electrospray, that does not generate multiply charged ions.

APCI ionization also called “hard ionization” operates at higher temperatures then ESI and with the help of a corona-discharge needle, plasma is created at the end of the metal capillary. In this plasma protons transfer reactions involving the solvent and fragmentation can occur.

APCI source is commonly used to analyze smaller, thermally stable polar and non-polar compounds. Like the ESI source, it can generate both positive and negative ions, and ion polarity can be switched on a spectrum-to-spectrum basis (Fig.4.4).

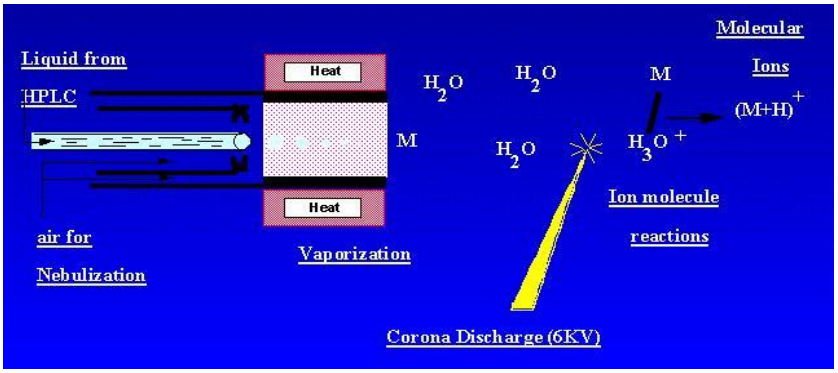


Fig.4.4- APCI Ionization Process (Source: http://www.cif.iastate.edu/mass-spec)

The choice of a ***Mass Analyzer*** is addressed by the application in intended for, the budget available and the performance desired. UniMS Lab was equipped with two different mass analyzers, the linear/3D ion trap and the TOF analyzers.

Linear/3D ion traps are mass analyzers whose operation is based on ion motion in electric fields.

The linear Ion Trap consists of a linear array of four symmetrically arranged rods where voltages are supplied. Forces are exerted in a plane normal to the direction (z-direction) in which the ions drift through the array in their journey from the ion source to the detector.

In the three dimensional analyzer, ions are subjected to forces applied by an field but the forces occur in all three dimensions, instead of just two. Hence, ions are trapped within the system of three electrodes- a ring electrode and two end-cap electrodes of hyperbolic cross-section.

The main difference between these two analyzers is that the linear ion trap has a higher ion capacity. The trapping mechanism and available scan types are similar, but the device volume is higher resulting in higher storage capacity and an increased linear dynamic range without the danger of the space charge distortions of the trapping characteristics of the ion trap (Fig.4.5).

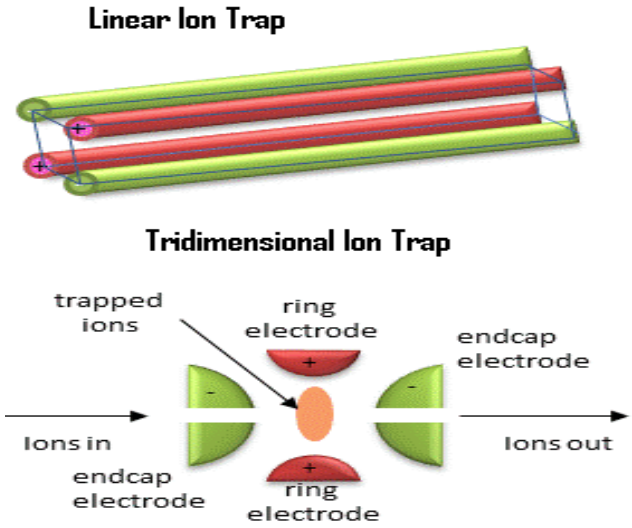


Fig.4.5- Linear/3D Mass Analyzer (source: http://csbsju.edu.html)

After generation of ions in ESI source, they are injected on the ion trap analyzer. Ions are trapped by applying a voltage to the ring electrode and then cooled to the middle of the trap due to the presence of helium as dampening gas. A selective instability is performed such that ions are ejected towards the detector in order of increasing *m/z*. Ejected ions are detected and the process is repeated. The mechanism of tandem mass spectrometry experiments, consist on the isolation of the selected precursor ion into the trap, while all other ions are expelled. Then collisions between the analyte ions and the helium present in the trap (now working as collision gas) are promoted, resulting in the dissociation of precursor ion. Product ions are scanned out sequentially to produce the MS/MS spectrum of that particular precursor ion.

The physical principle of Time-Of-Flight (TOF) ***Mass Analyzer*** is quite simple. When ions of different m/z are all given identical kinetic energies, the lighter ions will move faster, and thus, will have shorter flight times between the source and the detector. The ions are extracted from the ion source after a very short delay, which helps the decrease the initial distribution of kinetic energies since ion motion is dampened by collisions prior to extraction. This approach is used mainly with MALDI ionization. The main advantage of TOF analyzers is their theoretically unlimited mass range. Here, the high mass cutoff depends only on how long we wait until ions reach the detector

In order to obtain higher resolution results, working with a reflectron TOF analyzer is a beneficial possibility when compared with the linear TOF analyzer. The reflectron (often called an ion mirror) is a system that unifies the kinetic energy distribution, *i.e.* two ions with identical *m/z* values but with different kinetic energies, due to the initial energetic distribution, will arrive at the detector at the same time with the help of the reflectron (Fig.4.6).

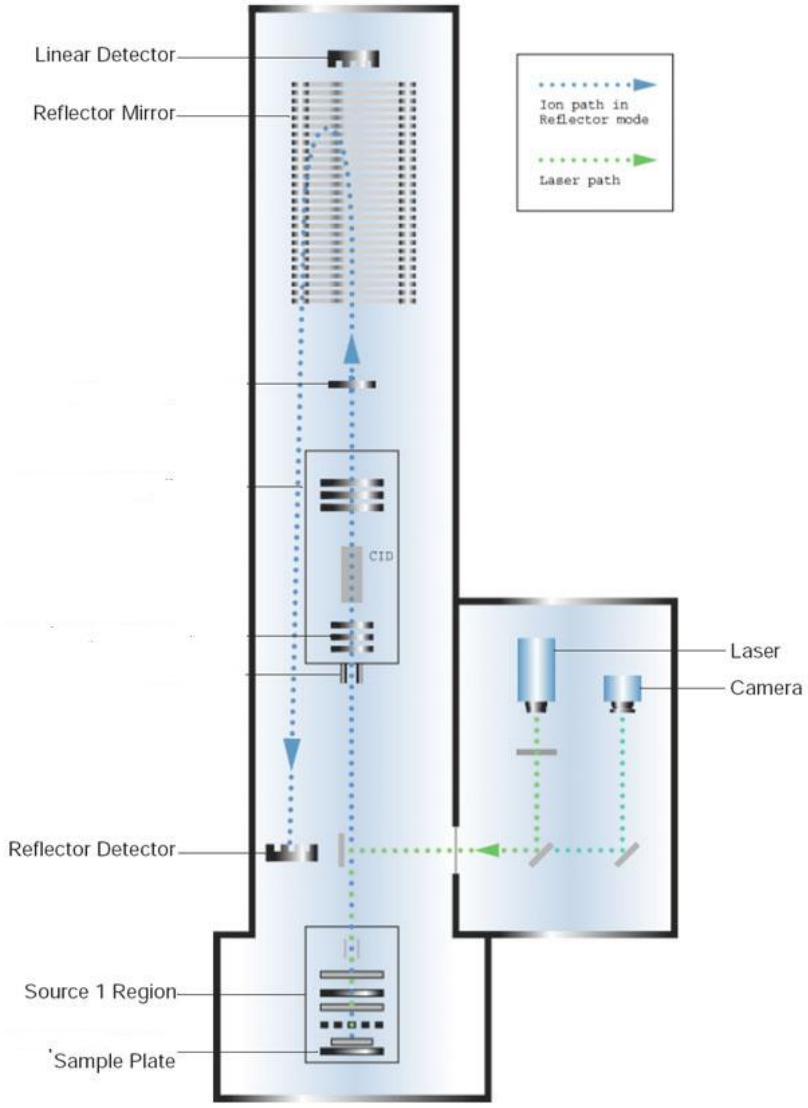


Fig.4.6- Linear and Reflectron TOF analyzer
(Source:http://proteo.cnb.csic.es/proteomica/malditoftof.jsp)

Mass spectrometry is now used across a broad range of disciplines and settings, including academic research, biotechnological development, pharmaceutical discovery, clinical testing, and environmental analysis, in the UniMS and earlier MS Lab Services, one of the most important field of study is Proteomics.

Proteomics is the study of all proteins in a biological system (*e.g.* cells, tissue, and organism) during specific biological events. Although it complements genomics, proteomics is considerably more difficult to study than genomics or even transcriptomics because of the dynamic nature of expression of proteins. Additionally, the majority of proteins undergo some form of post-translational modification (PTM), which further increases the proteomic complexity. The broad scope of proteomics has only begun to be realized within the last 15 years mainly due to technological developments in mass spectrometry.

The major customers using the services provided by UniMS can be divided into three diferent groups: internal academic research; external academic and public institutions and industry.

4.4 Professional Skills on Mass Spectrometry Services

Date: From April 2013 until present.

My collaboration with the UniMS comprises a range of responsibilities:

Managing the material and reagents necessary to the UniMS

A stock of specific MS and HPLC material is necessary to be maintained, in order to obtain a good workflow in MS assays, as well as a stock of standards and reagents required for specific and standard analysis.

Coordination of the entire customer service process

The client delivers the samples to the UniMS lab with the requested form. My responsibility process starts with the identification of the most appropriate methodology to be used for the specific samples. After analysis of the samples, a report is prepared and sent to the client, as well as the invoice for the assays performed.

Technician responsible for the verification, calibration and maintenance of mass spectrometers and HPLC systems

I was responsible for the quality control and maintenance of the UniMS' mass spectrometers and HPLC. Every six months the mass spectrometers and HPLCs are cleaned and then two types of process qualification / verification were being carried out:

(i) Performance Qualification (PQ), allowing the system to be verified as a whole and (ii) Operational Qualification (OQ) allowing the confirmation if each module is functioning properly.

Training of new users in the areas of mass spectrometry, chromatography and proteomics methodologies

One of the aiming of UniMS services is to make the equipment accessible to independent users, in order to carry out their research project goals. This will require training on the rules for the use of the respective equipments.

A more specific training is related to users who wish to receive basic training on LC-MS-based methodologies and in sample preparation for mass spectrometry analysis, in these cases the training is based on the development of LC-MS assays related to their project.

Implementation and elaboration of assays provided in the UniMS services

The laboratory performs development and validation of analytical methods as well as routine analysis for a broad range of chemical compounds, from small organic and organometallic compounds to peptides, oligosaccharides, nucleotides and proteins.

One of the main line of analyses performed in UniMS services is the one that refers to Proteomics. This includes the identification of proteins of samples derived from tissues, cells, bacteria, viruses, etc. whose workflow follows the following steps (Fig.4.7):

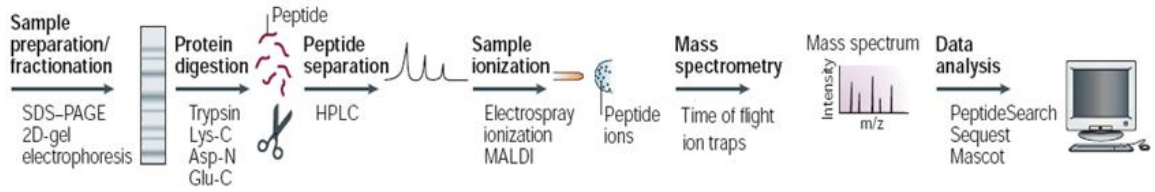


Fig.4.7- Workflow of a protein analysis (Adapted from: Steen H. and Mann M. (2004) The ABC's (and XYZ's) of peptide sequencing. Nature Reviews 5:699-711)

I have had the responsibility for the coordination and elaboration of the following analyses: (Table 4.1).

Table 4.1- Services and Methodologies provided in UniMS Services

Services	Methodology
Protein Identification; Proteome analysis	(nanoLC-Spotter)-MALDI-TOF/TOF nanoLC-ESI-MS/MS
Intact mass determination; Fragmentation Profile (MS^n); studies of stability product	MALDI-TOF/TOF LC-ESI-MS/MS
Purity profile	MALDI-TOF/TOF
Separation, Identification of small molecules and metabolites; Monitoring progression of enzymatic reactions	LC-ESI-MS/MS
Characterization of natural compounds	LC-APCI-MS/MS

Development, implementation and validation of LC-MS studies in order to quantify, identify and characterize biological compounds of samples from clinical and pharmaceutical industry origin.

Liquid chromatography-mass spectrometry (LC-MS) can be applied to a wide range of biological molecules and plays an important role in several areas of clinical biochemistry as well as is replacing other popular biochemical techniques such as immunoassays. The use of tandem MS in conjunction with stable isotope internal standards allows highly sensitive and accurate assays to be developed. However, high instrumentation complexity and the lack in universal methods are drawbacks that need to be overcome. Thus, method optimization is regularly required. During my professional career development I have developed method optimization for mass spectrometry-based proteomics with a large range of different samples, such as organic and inorganic molecules, proteins, peptides, lipids, bacteria, virus, parasites, ionic samples and dyes.

According to the analyte and sample as well as the questions to be answer, an LC-MS-based assay has a significant number of conditions and parameters that need to be taken into account and optimized. One of the most important parameters is the correct chromatographic column selection (reverse phase, normal phase, HILIC, etc.). Then the following variables must be optimized: (i) flow rate, (ii) mobile phase, (iii) injection volume, and (iv) column temperature. Optimized ionization MS parameters and collision energy are also evaluated by direct infusion of the sample in the MS.

The MS technique has a disadvantage called “ion suppression”. This form of interference occurs when other analytes than the targeted one cause a decrease in the signal of the analyte of interest. Compounds that are known to cause ion suppression include phospholipids, salts, drugs, metabolites, proteins and buffers. Therefore, method development in LC-MS-based require always ion suppression studies. Other factors can influence the LC-MS method development, such as low amount of sample, sample complexity, analyte and sample stability and instrumental limitations are just a few examples.

I have been the responsible for method development in the UniMS since the opening of this unit. Related to this responsibility I would like to highlight the research published in the Journal of medical Chemistry, where a novel class of falcipain inhibitors was studied, peptidomimetic molecules and pyrimidine nitriles **[A2].**

Pyrimidine tetraoxane hybrids displayed potent nanomolar antimalarial activity against three strains of *Plasmodium falciparum*. These compounds are reductively activated in the presence of high concentrations of iron (II) accumulated inside the parasite food vacuole after the digestion of large quantities of host´s hemoglobin. A reaction was intended to simulate the intraparasitic endoperoxide iron (II) that mediated the bio-activation process in the digestive vacuole of the parasite. In order to confirm the proposed mechanism, an LC-MS experiment was done to monitor the kinetics of the decomposition of tetraoxane-pyrimidine nitrile.

By request of the pharmaceutical industry, other two methods are still under development in the unit, as explained below.

One of them is a method for phospholipid identification and quantitation, with the aim to understand if there is a correlation between host-cell plasmatic membranes composition and virus envelopes. This is because the structural and functional diversity of lipids accounts for their involvement into a wide range of homeostatic processes and disease states, including lifestyle-related diseases as well as genetic conditions. The challenges presented by this diversity have been addressed to a great extent by the development of lipidomics, a platform that makes possible the detailed profiling and characterization of lipid species present in any cell, organelle, tissue or body fluid, and that it allows for a deeper understanding of the biological role of lipid networks.

The other LC-MS/MS method under development and implementation was requested in collaboration between the ITQB and IGC Institutes, and is described below.

Quorum sensing is a type of bacterial cell-to-cell signaling that allows for cell density dependent regulation of gene expression. Many of the behaviors mediated by quorum sensing are critical for bacterial colonization or infection, and autoinducer-2 has been proposed as a universal interspecies signaling molecule that allows multispecies colonies of bacteria, *e.g.*, biofilms or dental plaque, to behave as pseudomulticellular organisms. Liquid chromatography-tandem mass spectrometry technique was applied to the detection of autoinducer-2 from *Escherichia coli* and *Vibrio harveyi* in proof-of-concept studies and was then used to directly measure the concentration of the signal produced by oral bacteria in human saliva.

Date: From July 2003 until April 2013.

Since July 2013 I worked as Senior Technician at the Mass Spectrometry Laboratory. Some of my main activities as described earlier in this report were initiated in this laboratory under the guidance of Dr. Ana Varela Coelho, as described below.

Responsible for training new members in the GLP rules and elaboration of SOP's for new methodologies or new equipments

ITQB´s Mass Spectrometry Laboratory started providing service in 2003 and during this year was also included in the Analytical Services Unit (ASU) of ITQB/IBET. During this period, the ASU was certified by INFARMED (Portuguese Pharmacy and Medicine Agency) as compliant with OCDE Good Laboratory Practice (GLP), The MS Lab always worked in accordance with the GLP Rules.

Members outside the unit, such as MSc students, PhDs, had to receive mandatory training on the rules and general laboratory procedures and work under supervision until ensured they were complying with GLP norms. This task was under my direct supervision.

Elaboration of SOP´s for new methodologies according to FLP rules and for new equipments acquired for the mass spectrometry laboratory was other task under my responsibility.

Teaching in master and post-graduate classes on protein identification using mass spectrometry (practical classes)

Collaboration in practical classes taught at ITQB, according to the module protein identification using the mass spectrometry facilities was also done in a total of 8 courses.

Orientation and teaching of new students and technicians of mass spectrometry laboratory

Over the ten years I have been working at the MS lab , where my role on teaching focused mainly on practical guidance of master and PhD students, which were under the supervision of Dr. Ana Coelho, regarding sample preparation and purification and results analysis using a range of software tools.

Organization and participation of scientific events held at ITQB

The three training courses held at ITQB: (i) Practical course: Identification of proteins using Mass Spectrometry data, 26-27th January 2005; (ii) First RNEM course on Protein identification by Mass Spectrometry, 27-28th January 2010 and (iii) Hands-On course on Protein Identification by Mass Spectrometry, 2-4th November 2011, were dedicated to researchers using or willing to use mass spectrometry methods for protein identification. The mains objectives of these courses were to give practical insight into the most used MS strategies for protein identification, provide hands-on experience on crucial areas, and sample preparation and data evaluation..

Optimization, Development, and implementation of some LC-ESI/APCI-MS/MS assays, which I put into practice

I collaborated in the development of a MALDI-TOF method for analysis of porphyrins without using MALDI matrix with the group of organic chemistry from IST.

In collaboration with the ITQB's molecular thermodynamics group, we decided to put into practice the idea of using IL´s as matrices for MALDI .Ionic liquids (IL) are salts that have a melting point at or below 100 °C and possess negligible vapor pressure. IL matrices (ILMs) are easily prepared and require no crystallization with the analyte, which prevents "hot spots" and thus provides better shot-to-shot and spot-to-spot reproducibility. This higher reproducibility is crucial for quantitative analysis. These ILMs are organic salts prepared by equimolar mixtures of crystalline MALDI matrices like α-

cyano-4-hydroxycinnamic acid (CCA), 2,5-dihydroxybenzoic acid (DHB) or 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, SA) with organic bases.

The Unfolded Protein Response (UPR) is composed by homeostatic signalling pathways that are activated by excessive protein misfolding in the endoplasmic reticulum (ER). Ire1 signalling is an important mediator of the UPR, leading to the activation of the transcription factor Xbp1.

Ire1 mutant retinas have higher mRNA levels for targets of regulated Ire1-dependent decay (RIDD), including for the fatty acid transport protein (fatp). Importantly, down-regulation of fatp by RNA interference rescues the Rhodopsin-1 delivery defects observed in Ire1 mutant photoreceptors.

My task in the research related to the described UPR was to develop a method to quantify the phosphatidic acid levels existing in Ire1 mutant retinas. The results obtained allowed the publication of an article in cell report. **[A3]**

The use of Moloney murine leukaemia virus (MoMLV) derived retroviral vectors in gene therapy requires the production of high titter preparations. However, obtaining high titters of infective MoMLV retroviral vectors is difficult due to the vector inherent instability. The decrease in the cholesterol to phospholipids ratio in the viral membrane seems to be the major reason for the increased vector stability. Lipid extractions for cholesterol and phospholipid analyses were performed at the Animal Cell Technology lab from the ITQB.

My participation in this study was to develop a lipidomic method to analyse phospholipids composition, type and fatty acid content ratio in the viral membrane. The results of this assay allowed the publication of an article in the reviews Biotechnology and Bioengineering **[A7]**, a poster presentation **[P6]** and the participation in a conference in Stockholm **[C6].**

Methods demanded from the pharmaceutical industry done according to GLP norms

Since 2006 I participated in different request by pharmaceutical industry. All these studies were effectuated according to GLP rules.

During three years, from January 2008 to July 2010, I was responsible for validating a method, previously developed, to be used for the quantification of an active drug in a culture medium generated in permeability experiments. A full validation of the following parameters was performed: specificity/selectivity, accuracy, precision, recovery, linearity and the stability of test substance in apical and basolateral medium in the specific working range. After the validation was accomplished samples from Caco-2 epithelial cell permeability studies were analyzed using the validated method for quantification of the drug.

A Bio stability study of a protein linked to vaccine demand of the pharmaceutical industry was conducted at the MS lab for three years, where techniques such as 1D-PAGE, western blot and MALDI-TOF/TOF were used. In the beginning of this study, my task was just to collect MALDI-TOF/TOF data, but during the course of the study I became the technician responsible for almost all the experiments, since sample reception until the elaboration of reports. The idea it was to study the stability of a specific protein along time, in order to establish the shelf-life of this vaccine.

Another study was related to show that three drug induction assays with HepaRG cells were transferrable to naïve laboratories; in that study a LC-ESI-MS/MS methodologies were developed and optimized in order to quantify the drug metabolites excreted by the host cells.

4.5 Professional Skills on Mass Spectrometry Research Projects

Date: 2012 to 2014

The Rickettsiales *Ehrlichia ruminantium* (ER) is a small (0.2–2.5mm), Gram-negative obligatory intracellular bacterium transmitted by Ambyomma ticks and the causative agent of heartwater, a fatal tick-borne disease of ruminants in sub-Saharan Africa and in the Caribbean. Over the past 50 years, the only commercially available vaccination procedure was based on the controlled infection of animals with cryopreserved ER infected sheep blood, followed by antibiotic treatment. Although several vaccine candidates have been developed and evaluated (1), the development of a fully effective vaccine has been hindered by the difficulty of finding protective antigens against the pathogen. This is due to the large antigenic diversity of strains and to the lack of knowledge on ER biology and pathogenesis.

Within the host endothelial cells, ER presents a complex life cycle with two distinct developmental forms: an intracellular replicative reticulate body and an extracellular infectious elementary body responsible for infection. The successful establishment and maintenance of ER infection during its development inside the host cell depends on the bacteria's capability to subvert the host cell's defence response and successfully survive, proliferate or persist within the infected cell, using host resources for its own need.

My first task in this project “Comparative proteomic analyses between virulent and attenuated *E.ruminantium*: identification of potential antigens for a subunit heartwater vaccine” was to make a proteomics comparative analysis between virulent and attenuated forms of *E. ruminantium* for the identification of potential antigens for a vaccine against heartwater, using nanoLC-MALDI-TOF/TOF. Herein, ER was harvested from infected host bovine aorta endothelial cells (BAE) monolayers, purified by a multistep centrifugation process and total ER protein extracts were prepared using sonication, after optimization of the protein extraction protocol. Differentially expressed proteins were analysed by DIGE and 1D electrophoresis followed by microLC-MALDI-TOF/TOF analysis for total proteome characterization. Protein identification was performed by database searching.

Our major goal was to identify amongst the differentially expressed proteins in both phenotypes some antigens with potential to obtain a protective cellular immune response in order to develop a more effective immunization strategy against heartwater. At present, the infectious ER is being fully characterized by IBET, ITQB, IICT and CIRAD teams using transcriptomics and proteomics approaches.

This project allowed me to participate in a Short-Term Scientific Mission (STSM). The aim of this STSM was to relatively quantify the proteome changes of BAE's during infection using nanoLC-MS/MS. The STSM was supported and developed by the Mass Spectrometry Laboratory -Groupe Interdisciplinaire de Génoprotéomique Appliquée, University of Liege, Belgium and by the ongoing financed project ER-Transprot, ITQB/IBET, Portugal. BAE cells infected with ER and non-infected were treated by biotinylation approach. This approach consists of bonding of surface proteins to biotin molecules followed by their enrichment, identification and relative quantification between two biological states.

My main task was to analyze for the first time the effect of ER on the proteome of host bovine aorta endothelial cells (BAE), at the bacteria's mid-exponential phase of development (72 hours post-infection). For this, it was necessary: 1) to identify proteins differentially expressed between non-infected and ER-infected BAE cells using quantitative proteomics in total proteins extracts and 2) to identify surface proteins differentially expressed between non-infected and ER-infected BAE on biotinylated proteins extracts.

This 2-month STSM allowed me to extend to the Farm Animal field a comprehensive and efficient method developed at the University of Liege allowing identification and quantification of potentially accessible surface proteins. Furthermore, this STSM has allowed a collaboration between the two Proteomic/Mass Spectrometry Groups, GIGA (Univ Liége) and MS (ITQB), both with a track record on Farm Animal Proteomics and also has led to win a scholarship funded by the European Cooperation in Science and Technology (e-COST). **[A5, F, O2, O3, P1, P2, P4, C1]**

Date: 2012

My task in the project entitled "Iminoboronates: A new strategy for reversible protein modification", was to study new strategies for the modification of proteins with inclusion of boron, using the methodologies MALDI-TOF/TOF and direct infusion by electrospray. The results obtained from this study were published in the Journal of the American Chemical Society **[A6].**

Date: 2011 to 2013

One of the goals of the Plant Cell Biotechnology Laboratory was to develop a model to introduce and to study the expression of genes related to water deficit tolerance. A method for extraction and detection of trehalose-6-phosphate (T6P) in *Medicago truncatula* extracts was explored. Three extraction protocols - ethanolic, acid, and liquid-liquid - were tested in order to determine which would

have the capacity to recover a greater percentage of phosphorylated sugars. A hydrophilic interaction chromatography (HILIC) coupled to negative ion mode electrospray ionization with an ion trap mass spectrometer was implemented in order to quantify trehalose-6-phosphate (T6P), but the LC-MS detection of standard solutions showed that with this method T6P could not be separated from its isomer sucrose-6-phosphate (S6P). Thus, two-step enzymatic processes were designed. Invertase and trehalase are two enzymes that were assayed to assess whether they would specifically hydrolyze one of the isomers. Invertase was proven to hydrolyze specifically sucrose molecules whereas trehalase only hydrolyzed trehalose molecules. The ability of alkaline phosphatase (AP) to dephosphorylate unspecifically S6P and T6P, lead to the assumption that T6P could be quantified by dephosphorylation of plant extracted metabolites with AP followed by specific hydrolysis with either invertase or trehalase, using suitable blank controls.

Another methodology using HILIC-MS/MS was developed in order to separate the isomers T6P and S6P without having to use the previous approach with several steps of extraction and enzymatic separation. The method was optimized by testing eluent pH, type of organic solvent and alkalinizer, and gradient conditions. A specific column was tested. Optimization technique remains under investigation.

Date: 2010 to 2013

A project in collaboration with the Chemical Structural Center from Instituto Superior Técnico and with the Faculty of Medical Science from Universidade Nova de Lisboa entitled “Protein Adducts As Prospective Biomarkers of Nevirapine Toxicity” was performed in the lab MS.

Nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor used against human immunodeficiency virus type-1 (HIV-1), mostly to prevent mother-to-child HIV-1 transmission in developing countries. Despite its clinical efficacy, NVP administration is associated with a variety of toxic responses that include hepatotoxicity and skin rash. Although the reasons for the adverse effects of NVP administration are still unclear, increasing evidence supports the involvement of metabolic activation to reactive electrophiles. In the present study, we investigated the nature and specific locations of the covalent adducts produced in human serum albumin and human hemoglobin by reaction *in vitro* with the synthetic model electrophile 12-mesyloxy-NVP, used as a surrogate for the Phase II metabolite 12-sulfoxy-NVP. Multiple sites of modification were identified by two different MS-based methodologies, LC-ESI-MS/MS and MALDI-TOF/TOF.

These two distinct methodologies, which in some instances provided complementary information, allowed the identification of multiple adducts involving cysteine, lysine, tryptophan, histidine, serine, and the N-terminal valine of hemoglobin. Tryptophan, which is not a common site of covalent protein modification, was the NVP-modified amino acid residue detected in the two proteins and consistently identified by both LC-ESI-MS/MS and MALDI-TOF/TOF.

After this preliminary study with *in vitro* samples, a study of Hepatic toxicity in HIV-infected individuals exposed to nevirapine in different doses using LC-ESI-MS/MS and MALDI-TOF/TOF was carried out. The results are still under interpretation.

Date: 2007 to 2013

One of the major projects of the MS lab. was the understanding of echinoderm regeneration through proteomics. Regeneration is a complex cellular process in which, rather than simply forming a scar following injury, the animal forms a new functional tissue. Regeneration is a widespread process among metazoa, although not uniformly. Planaria, starfish, and some worms can regenerate most of their body, whereas many other species can only regenerate parts of specific tissues or fail to accomplish a functional re-growth, as is the case of mammal's central nervous system (CNS). Proteomic-based approaches are being recognized as extremely useful for study of regeneration events, also because there is a relevant contribution of posttranscriptional processes that frequently involve the occurrence of a broad range of PTMs. Echinoderms, as invertebrate deuterostomes, have amazing neuronal intrinsic growth aptitude triggered at any time point during the animal lifespan leading to successful functional tissue re-growth. This trait is known to be in opposition to their mammal close phylogenic relatives that have lost the ability to regenerate their central nervous system.

I participated in the study of radial nerve cord protein phosphorylation dynamics during starfish arm tip wound healing events. Thus, one of the results is a review article about echinoderm regeneration based on proteomics studies **[A4],** another result was a book chapter entitled Tandem MS and NMR: an Efficient Couple for the Characterization of Saponins" **[B2].** Furthermore, some results were presented as communications in conferences in the form of posters **[P3, P5 and P4]**, and oral communications **[O1]**. One of these posters was awarded as best poster presentation **[AWP 1].**

5 Evolution of Professional Experience

The career I have developed so far allowed me to put into practice many of the themes and techniques learned during my Chemistry degree and gave me the opportunity to expand my knowledge to academic and pharmaceutical industry.

Being my first professional experience after finishing the degree in chemistry, the integration in a team that was about to start, allowed me to gain organizational capacity, creating team spirit and social skills.

The fact that I helped to create the mass spectrometry laboratory since the very beginning, allowed me to get valences in laboratory management, comprehensive knowledge on the different aspects of operating a research laboratory, as well as a facilities. Later, this proved to be essential in the subsequent development of my professional career.

Having worked all this time within my area of expertise has allowed me to obtain a consolidation of knowledge and experience greatly enlarged. Having worked all this time in ITQB allowed me to explore various areas of chemistry and biology, as well as allowed me to grow in terms of responsibilities.

The nature of the various projects I have been involved in, its interdisciplinarity and the fact that different entities were involved, has brought me the skills and the ability to work in different environments. Additionally, the various training activities allowed me to develop further skills, consolidating and enlarging my background in research to areas such as proteomics, genomics, and metabolomics.

Having worked on GLP system from the beginning in the mass spectrometry lab, forced me to be strictly organized and pragmatic in any test or task that had to plan.

The fact that the mass spectrometry laboratory in the year that I started working there, did not have anyone specialized in chemistry and mass spectrometry, gave me the opportunity to be able to learn more about the equipment, the possibility to follow the vendors technicians for the different mass spectrometers responsible for repair and maintenance, forcing me to study, learn and find out more if it was possible to put into practice several methodologies for the academic community and industrial companies.

Working with the pharmaceutical industry has allowed me to deepen my knowledge in team management, data analysis and above all gain knowledge on bioorganic chemistry.

Working with the pharmaceutical industry enabled me to perceive reality from a different point of view on how to deal with very strict and demanding deadlines, as well as to work under pressure.

Throughout these years I could successfully broaden my knowledge on different areas of my initial training, having contributed to the publication of several articles in different research areas (from pure chemistry to medicine) in peer-review international journals.

The result of my journey over the years gave me a perspective of what I would do in the near future, in other words, follow a PhD and have the possibility to specialize in a specific area, taking advantage of the knowledge acquired over these 11 years of experience.

6 Skills and Competences

6.1 Personal Skills and Competences

Mother tongue Portuguese

Other Languages (Self-assessment)	Understanding				Speaking				Writing	
	Listening		Reading		Spoken interaction		Spoken production			
English	C1	Proficient user	C1	Proficient user	C1	Proficient user	C1	Proficient user	C1	Proficient user
Spanish / Castilian	C1	Proficient user	C1	Proficient user	C1	Proficient user	C1	Proficient user	B2	Independent user
French	C2	Proficient user	C2	Proficient user	C2	Proficient user	C2	Proficient user	C1	Proficient user

6.2 Social Skills and Competences

I have demonstrated good reasoning ability and communication in my work, proven by the teaching, orientation and training experience as mentor of new students.

I adapt easily to different working groups, situations and to change, an example of this is the STSM that I participated in Belgium.

I have demonstrated teamwork and self-motivation during this almost 11 years at ITQB.

6.3 Organization Skills and Competences

I have the ability to manage and resolve conflicts.

I have a good understanding of task analysis and scientific projects that I was part of.

I have ability to establish contacts and relationships necessary for projects, knowledge of project design, planning and ability to optimize resources.

6.4 Computer Skills and Competences

Advanced skills on Microsoft Office™ (Word™, Excel™ and PowerPoint™).

Advanced skills on data acquisition and result analysis software's (Bruker Daltonics-Data explorer; LC-MS-Xcalibur; MALDI-TOF/TOF-Data explorer, MoverZ; Peak erasor; SpeClust, GPS; Peaks; Mascot; Bioworks; PLGS; Isoquant and IPA).

Skills on graphics and softwares for *in silico* digestion and mass spectrometry fragmentation data.

7 Additional Information

7.1 Orientation and Training Experience

Isabel Marcelino *"Identification of vaccine candidates against Ehrlichia ruminantium using high-throughput proteomic analysis: a complementary approach to genome-based strategies for an improved Heartwater vaccine development", training of nanoLC-Spotter-MALDI/TOFTOF, 2013.*

Pedro Alves*, supervised training Pos-Doc Student in HILIC-LC-MS/MS assays, 2013.*

Rita Morgado*, supervised training in the project: "Integration of transcriptomic, proteomic and metabolomics profiles to understand the role of T6P in the water deficit response and recovery in Medicago truncatula" using LC-MS approaches, 2013.*

Inês Martins*, PhD Student from Instituto Superior Técnico da Universidade de Lisboa, supervised training "Hepatic toxicity in HIV-infected individuals exposed to nevirapine using MALDI-TOF/TOF approaches", curricular unit "Advanced experimental techniques" from the PhD Program of IST, 2013.*

Amal Moumenne*" PhD student from Antilles/Guyane University, Guadeloupe, France supervised training "Characterization of the outer membrane proteome of virulent E. ruminatum (on behalf of Integrated Action CRUP), 2012.*

Luis Domingues *Master student, supervised training "The effects of cadmium induced stress on protein expression profiles of Nicotiana tabacum" Master on Cellular Biology and Biotechnology, Faculty of Science, University of Lisbon, 2012.*

Rita Laires *Master student from IST, supervised training "Identification and characterization of bioactive peptides from starfish cell free coelomic fluid", 2011.*

Sofia Rodrigues*, supervised training BI grant on behalf of Project of the National Network for Mass Spectrometry (FCT ref: Rede/1504/REM/2005), 2009.*

Rui Palhinhas*, supervised training BI grant on behalf of Project of the National Network for Mass Spectrometry (FCT ref: Rede/1504/REM/2005), 2009.*

Kamila Koci *Post-doc, supervised training "Development and validation of methods for the characterization and quantification of peptides", 2009.*

Filipa Blasco*, Bachelor degree student in Biochemistry from Faculdade de Ciências e Tecnologia (FCT-UNL), supervised training Summer course in news methodologies, 2008.*

Liisa Arike *PhD training on behalf of Eramus Program, University of Tallin, Estonia, supervised training "Evaluation of protein microwave digestion methods", 2008.*

Marta Mendes, *PhD student, supervised training "Analysis of changes in the host cell proteome during hepatitis D virus infection", IHMT-UNL, 2008.*

André Lopes*, Master Student from Faculdade de Ciências da Universidade de Lisboa (FCUL), supervised training "Optimization of MALDI-TOF conditions for the study of metalloproteins" 2006.*

***Nuno candeias**, PhD Student from Instituto Superior Técnico da Universidade de Lisboa, supervised training "Study of porphyrin derivatives using MALDI ionization", curricular unit "Advanced experimental techniques" from the PhD Program of IST, 2005.*

***Gonçalo Graça**, Faculty of Sciences, University of Lisbon, ITQB-UNL, supervised training of MALDI-TOF user, 2005.*

***Conceição Almeida**, ITQB-UNL, supervised training of ESI-MS/MS user, 2005.*

***Elsa Lamy**, PhD student Évora University, supervised training Proteomics orientation, 2004.*

***Catarina Franco**, ITQB-UNL, supervised training of ESI-MS/MS and MALDI-TOF user, 2004.*

7.2 Fellowships and Awards and Prizes

[F] Fellowship for the Short Term Scientific Mission (STSM) by interaction of the organism COST

Subject Title: ER_TRANSPROT-Ehrlichia ruminantium proteome analysis: an approach complementary to transcriptomics towards a better understanding of the pathogenesis and against heartwater vaccine development.

Budget: 2.500 €

Research was conducted at: LSM-GIGA-Proteomics, Professor E.De Pauw, in University of Liège, Belgium.

Award and Prize for the best poster award

[AW1] 2nd ICAP – International Congress on Analytical Proteomics, Ourense, Spain.

7.3 Scientific Publications

[A1] *Jaimie-Leigh Jonker; Florence Abram; **Elisabete Pires**; Ana Varela Coelho; Ingo Grunwald; Anne Marie Power ""Homology but low similarity in adhesive proteins of stalked and acorn barnacles", **PLOS One**, submitted in **2014 April.***

[A2] *Rudi Oliveira, Rita C. Guedes, Patrícia Meireles, Inês S. Albuquerque, Lídia M. Gonçalves, **Elisabete Pires**, Maria Rosário Bronze Jiri Gut Philip J. Rosenthal,Miguel Prudêncio, Rui Moreira,Paul M. O'Neill,Francisca Lopes "Tetraoxane-Pyrimidine Nitrile Hybrids as Dual Stage Antimalarials", **Journal of Medicinal Chemistry, 2014 May,** DOI: **10.1021/jm5004528***

[A3] *Dina S. Coelho, Fatima Cairrão, Xiaomei Zeng, **Elisabete Pires**, Ana V. Coelho, David Ron, Hyung Don Ryoo and Pedro M. Domingos "Xbp1-independent Ire1 signaling is required for photoreceptor differentiation and rhabdomere morphogenesis in Drosophila", **Cell Report, 2013 Nov.***

[A4] *Franco, C., Soares, R., **Pires, E**., Koci, K., et al. "Understanding regeneration through proteomics". **PROTEOMICS 2013 Feb; doi: 10.1002/pmic.201200397.***

[A5] *Franco, C., Soares, R., **Pires, E.,** Santos, R., Coelho, A.V. "Radial nerve cord protein phosphorylation dynamics during starfish arm tip wound healing events". **Electrophoresis 2012 Dec; doi: 10.1002/elps.201200274.***

[A6] *Soares R, Franco C, **Pires E**, Ventosa M, Palhinhas R, Koci K, Martinho de Almeida A, Varela Coelho A. "Mass Spectrometry and Animal Science: Protein identification strategies and particularities of farm animal species". **Journal of Proteomics. 2012 Jul ; 75(14): 4190-206.***

[A7] *Pedro M. S. D. Cal, João B. Vicente, **Elisabete Pires**, Ana V. Coelho, Luís F. Veiros, Carlos Cordeiro and Pedro M. P. Gois, "Iminoboronates: A New Strategy for Reversible Protein Modification", **Journal Of The American Chemical Society. 2012 Jun; 134 (24): 10299-305.***

[A8] *Coroadinha AS, Silva AC, **Pires E**, Coelho A, Alves PM, Carrondo MJ. "The effect of osmotic pressure on the production of retroviral vectors: enhancement in vector stability", **Biotechnology and Bioengineering. 2006 Jun; 94(2): 322-9.***

[A9] *M Helena Florêncio, **Elisabete Pires**, Ana L Castro, Manuel R Nunes, Carlos Borges, Fernanda M Costa. "Photodegradation of Diquat and Paraquat in aqueous solutions by Tittanium Dioxide: Evolution of Degradation reactions and Identification of Intermediates ", **Chemosphere. 2004 Apr; 55 (3): 345-55.***

[B1] *Soares R., **Pires E**., Almeida A.M., Santos R., Gomes R., Koci K, Franco C.F., Coelho A.V. (2011). "Tandem mass spectrometry of peptides". In: Tandem Mass Spectrometry (Eds) **Jeevan Prasain, InTech Publishers DOI: 10.5772/1327.***

[B2] *Rita Laires, Kamila Koci, **Elisabete Pires**, Catarina Franco, Pedro Lamosa and Ana V. Coelho (2013). "Tandem MS and NMR: an Efficient Couple for the Characterization of Saponins". In: Tandem Mass Spectrometry-Molecular Characterization; **Publishers DOI: 10.5772/56477.***

7.4 Conferences and Workshops

[C1] EuPA 2013, Scientific meeting, Saint-Malo, France, **October 2013.**

[C2] Cost meeting, Farm Animal Proteomics, Kosice, Slovakia, **April 2013.**

[C3] RNEM Course on protein identification by Mass Spectrometry, organized by Mass Spectrometry Lab, ITQB, Oeiras, Portugal, **November 2011.**

[C4] The 4th Portuguese Mass Spectrometry Meeting", Lisboa, Portugal, **December 2010.**

[C5] First RNEM Course on protein identification by Mass Spectrometry", organized by Mass Spectrometry Lab, ITQB, Oeiras, Portugal, **January 2010.**

[C6] 5th Congress of the Portuguese Proteomics Network-ProCura and 1st International Congress on Analytical Proteomics-ICAP, Costa da Caparica, Portugal, **September 2009.**

[C7] 1st Meeting for users in Proteomics, organized by ThermoFisher Scientific, Barcelone, Spain, **November 2007**.

[C8] 4th Annual Meeting of the Portuguese Proteomics Network (Procura) and 1st Meeting of the Portuguese National Mass Spectrometry Network (RNEM), Aveiro, Portugal, **November 2007.**

[C9] 29th International Meeting of "High Performance Liquid Phase Separations and Related Techniques", Stockholm, Sweeden, **June 2005**.

[C10] Varian Spectroscopy Seminar, organized by VARIAN, ITQB, Oeiras, Portugal, **June 2004.**

[C11] Meeting of Chemistry, 10th National Meeting SPQ, Aveiro, Portugal, **July 2002.**

[C12] 2nd National Meeting of Chromatography, organized by Portuguese Society of Chemistry, Torre do Tombo, Lisboa, Portugal, **December 2001.**

[C13] 3rd National Meeting of Analytical Chemistry, organized by Portuguese Society of Chemistry, Instituto Superior de Engenharia de Lisboa, Portugal, **November 2001.**

7.5 Oral Communications

[O1] Catarina Franco, Rita Laires, Kamila Koci**,** ***Elisabete Pires*****,** Renata Soares, Joana Martins, Vinicius Kuffer, Silvia Mercurio and Ana V.Coelho." Omics profiling in echinoderms tissue and organ regeneration" EuPA 2013, Scientific meeting, Saint-Malo, France, **October 2013.**

[O2] ***Elisabete Pires***, Isabel Marcelino, Nathalie Vachiéry, Thierry Lefrançois, Gabriel Mazzuchelli, Edwin De Pauw, Ana V Coelho. "Changes of bovine aortha endothelial cells (BAE) proteome upon *Ehrlichia ruminantium* infection" Cost meeting, Farm Animal Proteomics, Kosice, Slovakia, **April 2013.**

[O3] Isabel Marcelino**,** Miguel Ventosa, Ludovic Pruneau**,** ***Elisabete Pires***, Damien F.Meyer, André M. de Almeida, Bernard Mari, Thierry Lefrançois, Ana V. Coelho and Nathalie Vachiéry. "Omics approaches to study the Rickettsia *Ehrlichia ruminantium:* towards improved knowledge on Heartwater disease." Cost meeting, Farm Animal Proteomics, Kosice, Slovakia, **April 2013.**

[O4] ***Elisabete Pires***; Franco, Catarina F; A. V. Coelho. "Mass Spectrometry Lab at ITQB", - ProteoRed Meeting- General Meeting, Miraflores de la Sierra, Madrid, Spain, **September 2005.**

7.6 Poster Presentations

[P1] ***Elisabete Pires***, Isabel Marcelino, Nathalie Vachiéry, Thierry Lefrançois, Gabriel Mazzuchelli, Edwin De Pauw, Ana V Coelho. "Changes on host cell proteome upon infection with the *Rickettsiales Ehrlichia ruminantium*, EuPA 2013, Saint-Malo, France, **October 2013.**

[P2] M.Ventosa, ***Elisabete Pires***, A.M. Almeida, N. Vachiéry ,A. V. Coelho, I. Marcelino." Studying *Ehrlichia ruminantium* proteome: towards the improvement of heartwater vaccine" EuPA 2013, Saint-Malo, France, **October 2013.**

[P3] Franco, Catarina F; Rita Laires; Kamila Koci; ***Elisabete Pires***; Renata Soares; Joana Martins; Vinicius Kuffer; Silvia Mercurio and Ana V Coelho. "Omics profiling in echinoderms tissue and organ regeneration", EuPA 2013, Saint-Malo, France, **October 2013.**

[P4] M.Ventosa, ***Elisabete Pires***, A.M. Almeida, N. Vachiéry ,A. V. Coelho, I. Marcelino, "Comparative proteomic analyses between virulent and attenuated *E.ruminantium*: identification of potential antigens for a subunit heartwater vaccine", EuPA 2012, Glasgow, Scotland, **July 2012.**

[P5] Franco, Catarina F; Soares, Renata; ***Pires, Elisabete***; Santos, Romana; Coelho, Ana V. Differential phosphoproteome of the regenerating radial nerve cord of the sea star *M. glacialis*, 2nd International Congress on Analytical Proteomics, Orense, Spain, **July 2011.** *Best Poster Award*

[P6] ***Elisabete Pires***; A Coroadinha, S Santos; A V Coelho. "Phospholipid composition of total lipid extracts by electrospray-MS/MS", XIV Congresso Nacional de Bioquímica, Vilamoura, Portugal, **December 2004.**

[P7] ***Elisabete Pires***; Ana Luísa Castro; Manuel Rosa Nunes; Carlos Borges; M. Helena Florêncio e Fernanda M. Costa. "Photodegradation of Diquat and Paraquat in aqueous solution using titanium dioxide: evolution of the degradation reactions and identification of intermediates", Encontro Nacional da SPQ, Porto, Portugal, **December 2004.**

[P8] ***Elisabete Pires***; Gonçalo Costa; Ana V.Coelho. "Mass Spectrometry of Proteins", 1st Annual Meeting of Portuguese Proteomic Network-ProCura, Instituto Nacional de Saude Dr. Ricardo Jorge, Lisbon, Portugal, **November 2003.**

[P9] ***Elisabete Pires***; Ana Luísa Castro; Manuel Rosa Nunes; Carlos Borges; M. Helena Florêncio; Fernanda M. Costa. "Photodegradation of Diquat and Paraquat in aqueous solutions by titanium dioxide: evolution of degradation reactions and identification of intermediates",16th internacional Mass Spectrometry Conference, Edinburgh, Scotland, **September 2003.**

[P10] ***Elisabete Pires***; Ana Luísa Castro; Manuel Rosa Nunes; Carlos Borges; M. Helena Florêncio; Fernanda M. Costa. "Study of Photodegradation of Herbicides in aqueous solution by action of TiO2", XVIII Encontro Nacional da SPQ, Aveiro, Portugal, **April 2002.**

8 Annexes

Annexes I- Certificates proving the training courses and participation in onferences

EuPA EUROPEAN PROTEOMICS ASSOCIATION

7th EuPA Congress
Saint Malo France
14 - 17 October 2013
Palais du Grand Large

A SMAP Congress hosted by
sfeap
SFSM

Saint Malo, FRANCE, October 17th, 2013

This is to certify that **Elisabete PIRES**

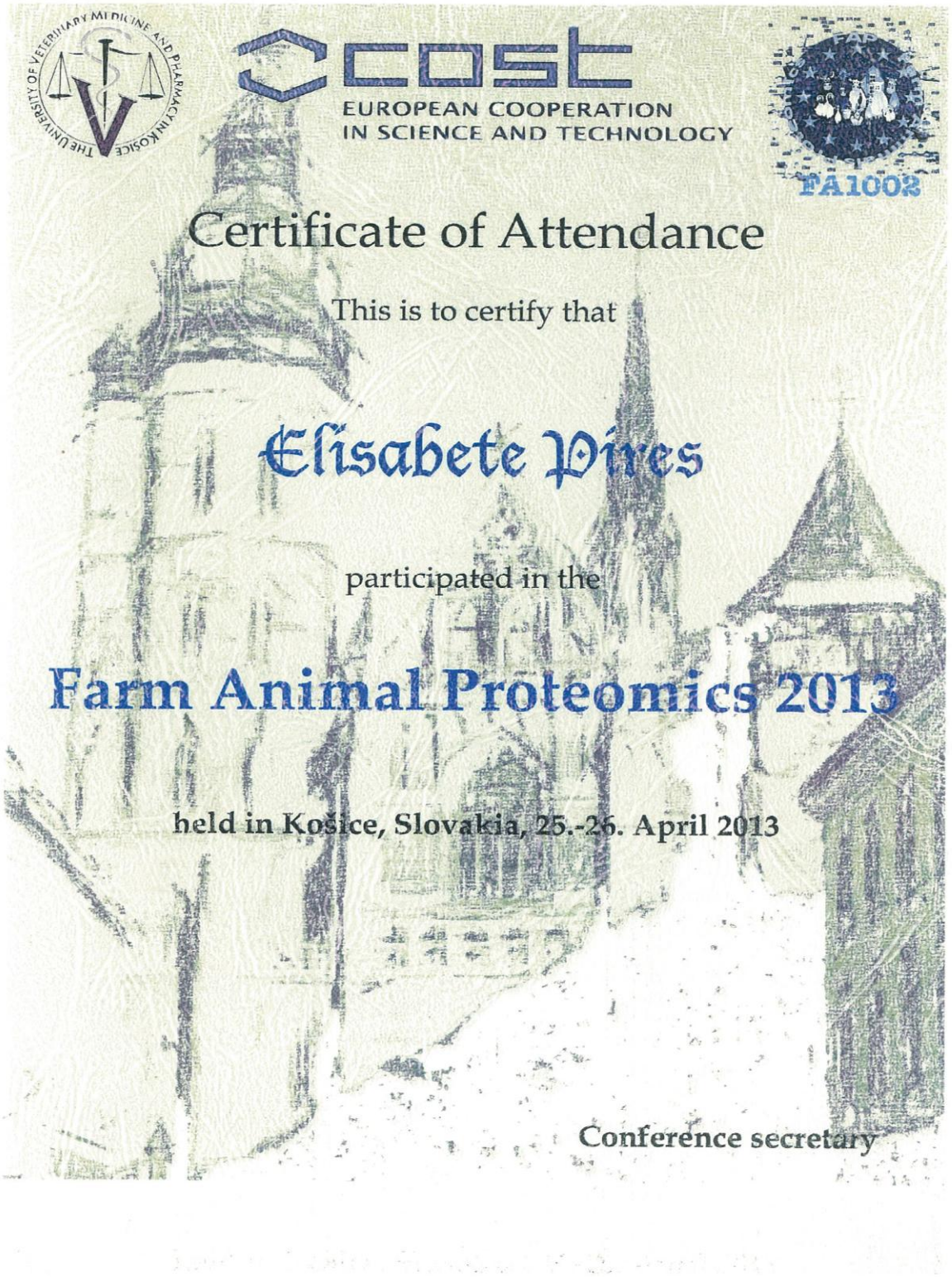
working for IBET

in **OEIRAS PORTUGAL**

attended to the EUPA 2013 Conference,

which was held in Saint-Malo, France, from October 2013, 14th to 17th.

Charles Pineau
President and Director Conference Chair


THE UNIVERSITY OF VETERINARY MEDICINE AND PHARMACY IN KOŠICE
cost
EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY
FA1002
Certificate of Attendance
This is to certify that
Elisabete Pires
participated in the
Farm Animal Proteomics 2013
held in Košice, Slovakia, 25.-26. April 2013
Conference secretary

COST Action FA1002 -Farm Animal Proteomics

Short-Term Scientific Mission Activity Report

Working Group 3 – *Advancing Methodology for Farm Animal Proteomics*

STSM Topic: Changes of bovine aorta endothelial cells (BAE) proteome upon *Rickettsiales Ehrlichia ruminantium* infection

STSM applicant: Elisabete Andrade Alves Pires

(Instituto de Técnologia Química e Biológica, ITQB, Oeiras, Portugal)

COST STSM Reference Number: COST-STSM-FA1002-10807

Host Institution: Groupe Interdisciplinaire de Génoprotéomique Appliquée (GIGA), Université de Liège, Liège, Belgium

Supervisor : Dr. Gabriel Mazzuchelli

Period covered by the report: **07.10.2012 to 7.12.2012**

iBET INSTITUTO DE BIOLOGIA EXPERIMENTAL TECNOLÓGICA

Av. República, Qta. do Marquês
2780-157 Oeiras - Portugal
Número de Identificação de Pessoa Colectiva 502 112 255

CERTIFICADO DE FREQUÊNCIA DE FORMAÇÃO PROFISSIONAL

Decreto Regulamentar Nº35/2002 de 23 de Abril

Certifica-se que **Elisabete Andrade Alves Pires** natural de**França**, nascido a **24/11/73**, nacionalidade, **Portuguesa** do sexo **Feminino**, portador do documento de identificação, nº **10440098** emitido por **Lisboa**, em **04/03/11**, frequentou em 09 e 14 de Fevereiro de 2012, com a duração total de 8 horas, o Curso de Formação Profissional

Boas Práticas de Fabrico de Medicamentos

Oeiras, 19 de Março de 2012

O Responsável pela Entidade Formadora

Certificado nº 06/2012

INSTITUTO DE TECNOLOGIA QUÍMICA E BIOLÓGICA /UNL

iBET

Certificate

Elisabete Pires

Organizing Committee

Training Course on Protein identification by Mass Spectrometry

RNE

ITQB | Oeiras | Portugal | 2-4 November 2011

Sponsored by FCT Fundação para a Ciência e a Tecnologia · Fundação Calouste Gulbenkian · applied biosystems · Waters THE SCIENCE OF WHAT'S POSSIBLE · Thermo SCIENTIFIC

DECLARAÇÃO

A Doutora Ana Maria Varela Coelho e a Doutora Elisabete Pires participaram, no ano lectivo de 2011/2012, na leccionação teórico-prática da disciplina de Métodos de Análise Molecular do Curso de Mestrado em Biologia Funcional do Instituto Superior de Agronomia.

Lisboa, 28 de Novembro de 2011

O Coordenador da Unidade de Crédito

(Ricardo Manuel de Seixas Boavida Ferreira
Professor Catedrático do Instituto Superior de Agronomia e
Investigador do Instituto de Tecnologia Química e Biológica)

iBET
Instituto de Biologia Experimental e Tecnológica

CERTIFICADO DE PRESENÇA

Elizabete Pires frequentou, no IBET/ITQB, a edição de 9-11 de Janeiro de 2008 da acção de formação interna em:

Quantificação da Incerteza em Ensaios Físico-Químicos

com a duração de 21 horas

O programa da acção de formação, lista de presenças e curriculos do formador serão mantidos no Arquivo da Unidade BPL do IBET pelo periodo mínimo de 5 anos.

Ricardo Bettencourt da Silva

ESCOLA DE SOCORRISMO
CRUZ VERMELHA PORTUGUESA

CERTIFICADO DE FORMAÇÃO PROFISSIONAL

Dec. Reg. Nº 35/2002

Certifica-se que ELISABETE ANDRADE A. PIRES natural de França, nascida a 24-11-1973, portadora do Bilhete Identidade 10440098, emitido por LISBOA em 04-01-2005, concluiu com aproveitamento, em 30-10-2007, o seguinte curso:

FORMAÇÃO BÁSICA DE SOCORRISMO

698/FOR/REC/2007

que decorreu de 29-10-2007 a 30-10-2007, com a duração total de 12 horas, tendo obtido a classificação final de 18 valores numa escala de 0 a 20.

Lisboa, 27-12-2007

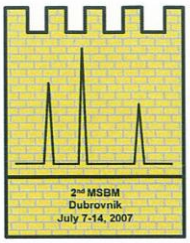
ESCOLA DE SOCORRISMO
CRUZ VERMELHA PORTUGUESA

O Responsável pela Entidade Formadora

ESCOLA DE SOCORRISMO
CRUZ VERMELHA PORTUGUESA

Certificado nº 6090/2007

ESCOLA DE SOCORRISMO DA CRUZ VERMELHA PORTUGUESA
Av. Almirante Gago Coutinho, 156 • 1700-033 LISBOA • Tel.: 218 459 440 • Fax: 218 459 458 • Contribuinte n.º 500 745 749



2nd Summer Course on Mass Spectrometry in Biotechnology and Medicine
Center for Advanced Academic Studies, Dubrovnik, Croatia
July 7-14, 2007

14 July 2007

Certificate of participation

Hereby we confirm that

Elisabete Pires

attended the 2nd Summer course on Mass Spectrometry in Biotechnology and Medicine, Center for Advanced Studies, Dubrovnik, Croatia,July 7-14,2007

Michael Mormann

Dr. Michael Mormann
(Organizing Committee, 2nd MSBM)

SPECANALITICA
equipamentos científicos, lda

UBiA

CERTIFICADO DE PARTICIPAÇÃO

Certifica-se que ***Elisabete Pires*** participou na acção de formação "Acção de formação sobre a acreditação de laboratórios e a NP EN ISO/IEC 17025:2005 3ªed.", de 13 a 16 de Março de 2007, com uma duração de 28 horas, organizada pela Specanalitica.

A coordenação

Os formadores

INSTITUTO DE BIOLOGIA EXPERIMENTAL E TECNOLÓGICA

IBET

CERTIFICADO DE PRESENÇA

Elizabete Pires frequentou, no IBET, a edição de 22 de Fevereiro de 2007 da acção de formação interna em:

Gestão documental, CAPA, incertezas e OOS na Unidade BPL

com a duração de 1 dia

O programa da acção de formação, lista de presenças e curriculos do formador serão mantidos no Arquivo da Unidade BPL do IBET pelo periodo mínimo de 5 anos.

Ana Luisa Simplício

SPECANALITICA
equipamentos científicos, lda

UBiA

CERTIFICADO DE PARTICIPAÇÃO

Certifica-se que *Elisabete Pires* participou na acção de formação sobre o **Cálculo de Incertezas em Laboratórios Químicos**, de 27 a 28 de Março de 2006, com uma duração de 14 horas, organizada pela Specanalitica e pelo UBiA.

A coordenação

Os formadores

Paula Alexandra Teixeira

CERTIFICADO DE FREQUÊNCIA DE FORMAÇÃO

Dec. Reg. Nº 35/2002

CRUZ VERMELHA PORTUGUESA

DIRECÇÃO DE ENSINO DE SOCORRISMO

Certifica-se que ELISABETE ANDRADE A. PIRES nascida a 24-11-1973, portadora do Bilhete Identidade nº 10440098 , emitido por LISBOA em 04-01-2005, concluiu com aproveitamento em 19-11-2005, o seguinte curso:

FORMAÇÃO BÁSICA DE SOCORRISMO

481/FOR/2005

que decorreu de 14-11-2005 a 19-11-2005, com a duração total de 24 horas, tendo obtido a classificação final de 18 valores numa escala de 0 a 20.

Lisboa, 09-01-2006

O Responsável pela Entidade Formadora

CRUZ VERMELHA PORTUGUESA · Direcção do Ensino de Socorrismo ·

Certificado nº 5487 / 2005

CRUZ VERMELHA PORTUGUESA - Jardim 9 de Abril, 1 a 5 1249-083 LISBOA
Tel: 213 905 571 Fax 213 953 064 Cont. nº 500 745 749

HPLC 2005
STOCKHOLM

The 29th International Symposium on High Performance Liquid Phase Separations and Related Techniques

CERTIFICATE OF ATTENDANCE

This is to certify that

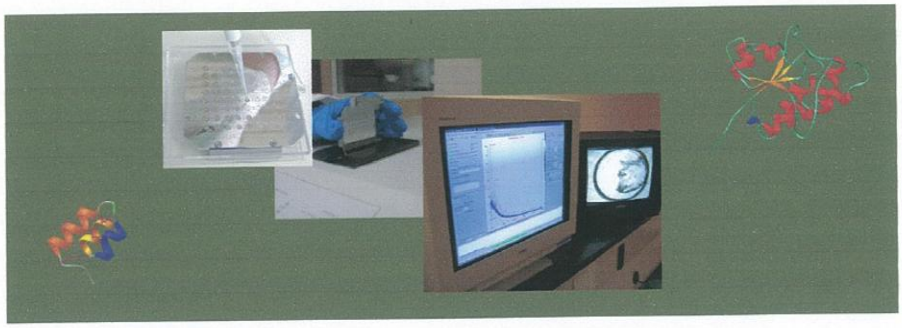
Elisabete Andrade Alves Pires

has attended the The 29th International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC) which was held in Stockholm, Sweden, June 26-30, 2005

Douglas Westerlund
President

Practical Course

Identification of proteins using Mass Spectrometry Data



Certificado de participação

Certifica-se que **Elisabete Pires** participou no curso prático "Identification of Proteins using Mass Spectrometry Data" que teve lugar no Instituto de Tecnologia Química e Biológica (ITQB-UNL), Oeiras, dias 26 e 27 de Janeiro de 2005

Ana Coelho

Prof. Doutora. Ana Coelho

ORGANIZATION

Laboratório de Espectrometria de Massa

Instituto de Tecnologia Química e Biológica

Instituto de Biologia Experimental e Tecnológica

Instituto Gulbenkian de Ciência

IN COLABORATION WITH

SEPROT Sociedad Española de Proteómica

Thermo Electron Corporation

UNICAM
Sistemas Analíticos, Lda.
GRUPO THERMO

CERTIFICADO

A **UNICAM** Sistemas Analíticos, *certifica que.*

Elisabete Pires

Participou no seminário sobre Cromatografia Líquida e Espectrometria de Massa (HPLC-MS/MSn) no ITQB, no dia 25 de Janeiro de 2005, das 9 as 17 H, com o seguinte programa:

9.00 - Confirmation of registration;
9.30 - Introduction to Mass Spectrometry and HPLC MSMSn;
10.15 - Method development in HPLC MS;
11.00 - Structural elucidation with multiple stages of fragmentation on linear ion trap;
11.20 - Quantitation using triple quadrupole technology;
11.40 - High resolution analysis on triple quandrupole;
12.00 - LTQ FT – Introducing the ultra high mass accuracy and resolution;
14.00 -Overview of strategies for protein identification with mass spectrometry;
14.45 - LC-MS/MS Specific Peptide Characterisations at high sensitivity by Ion Trap MS using SIR mode;
15.25 - LTQ - The instrumentation for fast, sensitive, and confident protein identification;
16.30 - LTQ FT in proteomics.

Palestrantes: Dra. Micaela Scigelova, Dr. Paul Humphrey, Dra. Anabela Marina, Eng. Bernabé Bodas e Dr. Daniel Ettlin.

Daniel Ettlin

Congresso Nacional de Bioquímica

CERTIFICADO

Certifica-se que *Elisabete Andrade Alves Pires*,

participou no XIV Congresso Nacional de Bioquímica que teve lugar em Vilamoura, de 2 a 4 de Dezembro de 2004.

A Presidente da Comissão Organizadora

XIV Congresso Nacional de Bioquímica - Vilamoura 2004 - SPB

Vilamoura, 2-4 Dezembro 2004

Sociedade Portuguesa de Bioquímica SPB

CDRH CONSULTORES ASSOCIADOS, LDA.

UNIÃO EUROPEIA
Fundo Social Europeu

PORLVT
Programa Operacional da Região de Lisboa e Vale do Tejo

Portugal em Acção

Certificado de Formação Profissional

(Dec.Reg.n.º 35/2002, de 23 de Abril)

CDRH – Consultores Associados, Lda.

Entidade Acreditada pelo INOFOR - processo nº 2355

Cont. 504 473 336 CAE 80421

Sede: Av. De Roma, n.º 62 – 5 Dto., 1700-349 Lisboa

Certifica-se que Elisabete Andrade Alves Pires, nascida a 24/11/1973, nacionalidade Portuguesa, portadora do BI n.º 10440098, emitido pelo Arquivo de Identificação de Lisboa em 22/09/1999, Contribuinte n.º 200726013, concluiu, o curso

Gestão de Projectos com o Microsoft Project

que decorreu de 01/09/2004 a 14/10/2004, com duração total de 90 horas, tendo obtido a classificação final de Muito Bom

CDRH - Consultores Associados, Lda.
Cont. 504 473 336
Av. de Roma, 62-5.º Dto
1700-349 LISBOA

Lisboa, 14 de Novembro de 2004

Certificado n.º 14/04/GPMP

Responsável pela Entidade

VARIAN

Inspiring Excellence

This is to certify that

Drª Elisabete Andrade Alves Pires

has attended

Varian Spectroscopy Seminar

Ross Ashdown

Presenter: Ross Ashdown
June 22nd, 2004

we innovate we respect we care we learn we deliver

CDRH CONSULTORES ASSOCIADOS, LDA.

UNIÃO EUROPEIA
Fundo Social Europeu

PORLVT
Programa Operacional da Região de Lisboa e Vale do Tejo

Portugal em Acção

Certificado de Formação Profissional

(Dec.Reg.n.º 35/2002)

CDRH – Consultores Associados, Lda.

Entidade Acreditada pelo INOFOR - processo nº 2355

Cont. 504 473 336 CAE 80421

Sede: Av. De Roma, n.º 62 – 5 Dto., 1700-349 Lisboa

Certifica-se que Elisabete Andrade Alves Pires, nascida a 24/11/1973, nacionalidade Portuguesa, portadora do BI n.º 10440098, emitido pelo Arquivo de Identificação de Lisboa em 22/09/1999, Contribuinte n.º 200726013 frequentou, o curso

Segurança, Higiene e Saúde no Trabalho

que decorreu de 28/04/2004 a 21/05/2004, com duração total de 32 horas, tendo obtido a classificação final de Muito Bom

Algés, 4 de Junho de 2004

CDRH - Consultores Associados, Lda.
Cont. 504 473 336
Av. de Roma, 62-5.º Dto.
1700-349 LISBOA

Certificado n.º 16/04/SHST

Responsável pela Entidade

FORQUAL
Recursos Humanos e
Qualidade Lda.

Certificado

Elisabete Pires

frequentou o "Curso de Estatística para Laboratórios"com a duração de 35 horas (5 dias), dado pela empresa de formação profissional e consultadoria FORQUAL LD., a pedido da Coordenadora dos Laboratórios do Instituto de Tecnologia Quimica e Biologia - ITQB .
O Curso foi realizado em Maio de 2004 nas instalações do ITQB em Oeiras

Lisboa 31 de Maio de 2004

Maria Cecília Moura da Silva
Gerente e Formadora da Forqual Ld

Rua Profª Virgínia Rau, 10 4°A- 1600 Lisboa Telefone ou Fax 217597691
Contribuinte n°502929456

INSTITUTO DE BIOLOGIA EXPERIMENTAL E TECNOLÓGICA

Certified GLP Unit

CERTIFICADO DE PRESENÇA

Certifico que **Elisabete Pires** frequentou a edição de 10 de Maio de 2004 da acção de formação interna com a duração de 2h30m em:

Boas Práticas de Laboratório
e a sua aplicação na Unidade BPL do IBET

O programa da acção de formação, lista de presenças e curriculos do formador serão mantidos no Arquivo da Unidade BPL do IBET pelo periodo mínimo de 5 anos.

Unidade de Garantia da Qualidade

Ana Luisa Simplício

Postal Address: Aptd. 12 - 2781-901 Oeiras - PORTUGAL
Access: Av. da República - Quinta do Marquês - Oeiras
Tels.: (351) 21 442 77 87 / 21 442 11 73 . Fax: (351) 21 442 11 61 . http://www.ibet.pt

CERTIFICADO DE PRESENÇA

Certificamos que

Elisabete Andrade Pires

frequentou a edição de

11, 12, 15, 16 e 17 de Dezembro de 2003

da acção de formação

Estatística Aplicada a Laboratórios de Análise-35H

RELACRE

Associação de Laboratórios Acreditados de Portugal

A DIRECÇÃO TÉCNICA

inofor

INSTITUTO DE BIOLOGIA EXPERIMENTAL E TECNOLÓGICA
IBET
Certified GLP Unit

Certificado

Certifica-se que **Elisabete Pires** frequentou com aproveitamento a formação interna "Validação de Métodos Cromatográficos" na qual foram referidos os seguintes temas:

- Avaliação da adequabilidade do sistema;
- Especificidade do método;
- Linearidade;
- Robustez;
- Homogeneidade de variâncias;
- Eliminação de valores aberrantes.

A formação teve lugar nos dias 20, 21 e 24 de Outubro de 2003 com a duração de 16 horas e foi organizada pelo Director Interino do Laboratório Analítico da Unidade BPL do IBET.

O Formador

INSTITUTO DE BIOLOGIA EXPERIMENTAL E TECNOLÓGICA (IBET)
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Engº António Ferreira

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DECLARAÇÃO

Foi ministrada informação à técnica de laboratório Elisabete Pires em 18 de Agosto de 2003 sobre metodologia BPL, nomedamente a publicação "OECD Principles on Good Laboratory Practice"-Paris 1998 e a norma Portuguesa EN ISO/IEC 17025, Instituto Português da Qualidade 2000 e apresentado o Manual de Qualidade da Unidade BPL do IBET. Demonstrou ter apreendido os conhecimentos relevantes ao desempenho das suas funções na Unidade BPL.

Ana Maria Varela Coelho
Responsável pela Sala de Espectrometria de Massa

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Certified GLP Unit

DECLARAÇÃO

Elizabete Pires recebeu formação no Laboratório Analítico do IBET situado no edifício da Química, em 30 e 31 de Julho, 1, 2 e 3 de Setembro de 2003 nas áreas que seguidamente discriminamos.

- 30 e 31 Julho de 2003

Introdução geral ao equipamento de HPLC. Procedimentos gerais para a realização de ensaios de validação para o equipamento de HPLC.

- 01 de Setembro de 2003

Verificação da balança analítica.

Verificação de material de vidro (provetas, balões volumétricos, pipetas). Referência às condições em que o material de vidro e a água necessária para a verificação devem-se encontrar para a realização da verificação.Abordou-se também a forma de se calcular os erros do material e respectivos critérios de aceitação. Referiu-se as causas que levam à degradação deste tipo de material e o que fazer quando o material não cumpre os critérios de aceitação do Procedimento Operativo.

- 02 de Setembro de 2003

Recebeu formação na área de HPLC, utilização de equipamento.

- 03 de Setembro de 2003

Avaliação da conformidade das micropipetas de acordo com o Procedimento Operativo 06LD:

- definição das condições de trabalho a seguir no controlo interno;
- definição das condições de trabalho a seguir no controlo interno;
- medidas correctivas a tomar em caso de não conformidade;
- avaliação técnica de um certificado de calibração externa segundo os critérios de aceitação para este tipo de calibração.

Demonstrou ter aprendido os conhecimentos relevantes nas áreas referidas.

António Ferreira
Direcção Interina do Laboratório Analítico

08.09.2003

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Annexes II- Reference Letter of Responsible from Mass Spectrometry Laboratory of ITQB

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Reference letter for Elisabete Andrade Alves Pires

6 June 2013

To Whom It May Concern:

Elisabete Andrade Alves Pires has integrated the team of the Laboratory of Mass Spectrometry of ITQB as a graduated technician in July 2003, having been promoted to senior technician in July 2008. The Laboratory of Mass Spectrometry as been implemented as Facility in 2003 and has join the Analytical Service Unit ITQB/IBET in 2006, which was certified in Good Laboratory Practices (GLP) in accordance with the OECD Principles. In this context has been provided scientific support to various research groups of Oeiras Associate Laboratory (ITQB / IBET / IGC), other academic institutions and industry, particularly to the pharmaceutical industry.

During this time Elisabete has been responsible for implementing and validating several mass spectrometry methods and performing assays involving LC-ESI-MS and MALDI-TOF/TOF of organic, inorganic and biological samples, particularly intact and digested protein. Experience has been gained from the use of mass spectrometers of different manufacturers, particularly Bruker, Thermo and ABSciex. Having demonstrated a great capability in its use and profound knowledge about their mode of operation, which allows a high capacity for problem solving. Additionally, Elisabete was the responsible for the assays performed on behalf of two Good Laboratory Practice studies requested by the pharmaceutical industry.

Elisabete had also a strong involvement in the implementation of the quality system in the Laboratory of Mass Spectrometry, performing the preparation and review of operating procedures. She has been responsible for verification, calibration and maintenance of various equipments, including two LC-ESI-MS systems, and has been involved in the daily management of the Laboratory. Furthermore

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collaborates in training of other technicians and students, as users of mass spectrometry equipment. It is also responsible for Laboratory Safety and has integrated the body of ITQB rescues. Performs with high responsibility and commitment all the tasks that have been requested and has expressed strong willingness to attend training courses that allow her to strengthen her technical and scientific knowledge.

For the last five years Elisabete has expressed great enthusiasm in participating in research projects under the responsibility of the Mass Spectrometry Laboratory. She was involved in six projects, from which the quality of the obtained experimental results has allowed the publication of three peer review research papers, two reviews and one book chapter. Recently, she has been intensely collaborating in the project "ER_TRANSPROT-Ehrlichia ruminantium proteome analysis: a complementary approach to transcriptomics towards increased knowledge on heartwater pathogenesis and vaccine development" PTDC/CVT/114118/2009, with the financial support of the Portuguese National Funding Agency (FCT). On behalf of this project a two months scientific mission was performed at the Mass Spectrometry Laboratory of Liége University, Belgium. This training period was fundamental for Elisabete since it allowed her to gain insights and experience with 2D-nano LC and high resolution mass spectrometry for the identification of proteins. An oral presentation on the results obtained was presented last April at the 3rd Farm Animal Proteomics Meeting, Slovakia.

In addition to the professional characteristics described above, Elisabete is a pleasant person that develops good relationships with the majority of her colleagues. All these reasons lead me to consider Elisabete an excellent technician that is quickly increasing her scientific capabilities to perform more demanding and high quality work. She is one of my team members that I am deeply sorry to lose.

Mass Spectrometry Laboratory Coordinator

Ana Maria Varela Coelho
Assistant Researcher

Annexes III- Statement from ITQB

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Knowledge Creation

DECLARAÇÃO

Para efeitos de entrega na Faculdade de Ciências e Tecnologia - UNL declara-se que: Elisabete Andrade Alves Pires, com categoria equiparada a Técnico Superior, celebrou com o Instituto de Tecnologia Química e Biológica António Xavier, no âmbito do Laboratório Associado os seguintes contratos:

- De 1 de Julho de 2003 a 30 de junho de 2008 (contrato de trabalho em funções públicas a termo certo).

- De 1 de Julho de 2008 até à presente data (contrato de trabalho a termo resolutivo incerto).

Teresa Venda

Administradora

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