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CEMUP CENTRO DE MATERIAIS DA UNIVERSIDADE DO PORTO | FC FACULDADE DE CIÊNCIAS UNIVERSIDADE DO PORTO

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Comparison study of LC Systems and Electrospray sources (ESI and nano-ESI) coupled to Orbitrap analyzer

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INTRODUCTION

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Understanding the role proteins play in different states of the body has significantly influenced innovation in proteomics based methodologies. Shotgun proteomics is one of the most commonly used techniques and is based on proteolytic digestion of proteins, resulting in peptides that are subsequently separated by liquid chromatography (LC) and analyzed by tandem mass spectrometry (MS^2) followed by bioinformatics interpretation. Proteomics depends upon MS and MS^2 for determining protein/peptide sequences and molecular composition. These studies typically involve LC coupled to electrospray ionization mass spectrometry (MS). Different segments of the proteomics platform (LC, ionization source and mass spectrometer) affect the quality of proteomics data. [1-6]

In this work we will present the results of proteolytic digestion of proteins obtained in the latest mass analyzer available in the market, Orbitrap analyzer, to investigate it´s sensitivity. For that, we performed a comparison study with two different liquid chromatography systems, HPLC Accela™ and Dionex Ultimate 3000 RSLCnano-LC system attached to two different ion sources, ESI and Nano-ESI, respectively.

MATERIALS AND METHODS

Sample Preparation: Pierce ™ Bovine serum albumin (BSA) was digest in–solution, with prior reduction and alkylation, accordingly to the *Instuctions Pierce Trypsin Protease, MS grade* of the applications note from Thermo Scientific.

Liquid Chromatography:

(1) Sample separation and the introduction of 10 µL of sample into the mass spectrometer was performed using a reversed phase high performance chromatography with a two component mobile phase system: 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile/H_2O (90:10) (B). Dionex Ultimate 3000 RSLCnano-LC system and 150mm x 75 µm ID (3µm particle size) Thermo Scientific™ PepMap™ Easy-Spray™ column were employed at a flow rate of 300 nL/min. Column temperature was set at 35ºC. Peptides were eluted using a gradient of 5% to 50% B over 57 minutes followed by 50% to 90% B over 2 minutes and 3 mnutes of 90% B.

(2) 10 µL of sample was injected using a reversed phase high performance chromatography with 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). HPLC Accela Pump™ 600 system and 50mm x 2.1 mm ID (1.7 µm particle size) Waters Acquity BEH column were employed at a flow rate of 200 µL/min. Peptides were eluted using a gradient of 2% B over 5 minutes followed by 2% to 55% B over 40 minutes and 10 mnutes of 90% B.

Mass Spectrometry: Data was acquired on the a LTQ Orbitrap XL™ hybrid mass spectrometer with Nano-EASY-Spray™ ion source (For LC-**A, fig.1**) or with electrospray ion source (for LC –**B, fig.2**) . The mass spectrometry was operated in the positive mode and ms survey scans were acquired at an Orbitrap resolution of 60 000 for an *m/z* range from 300-2000. Tandem MS (MS/MS) data were acquired using a data dependent method with dynamic exclusion: top 6 most intense ions were selected for collison induced dissociation (CID). CID settings was 35% of normalized collision energy. Data were record with Xcalibur software 2.1 and analyzed using SequestHT search for validation of protein identifications with Δm≤5 ppm (Proteome Discoverer 1.0, Thermo Scientific)

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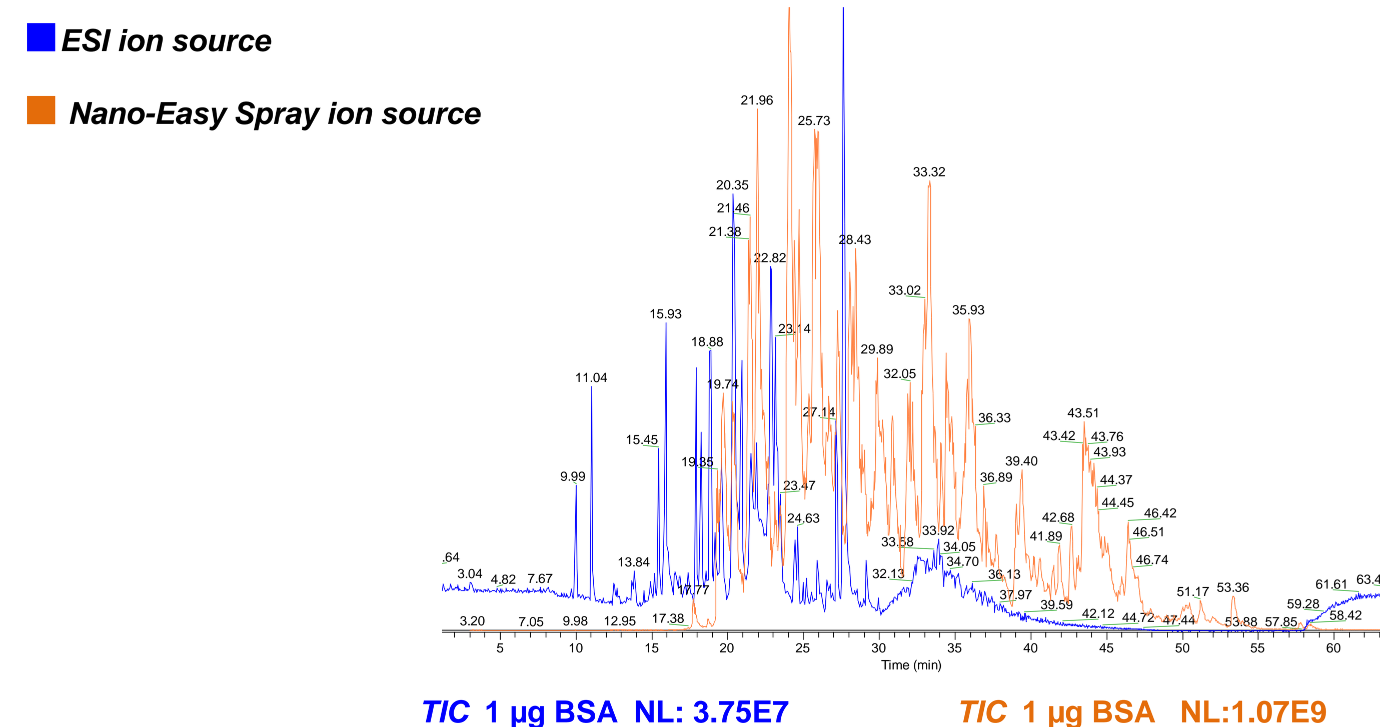
Acknowledgments

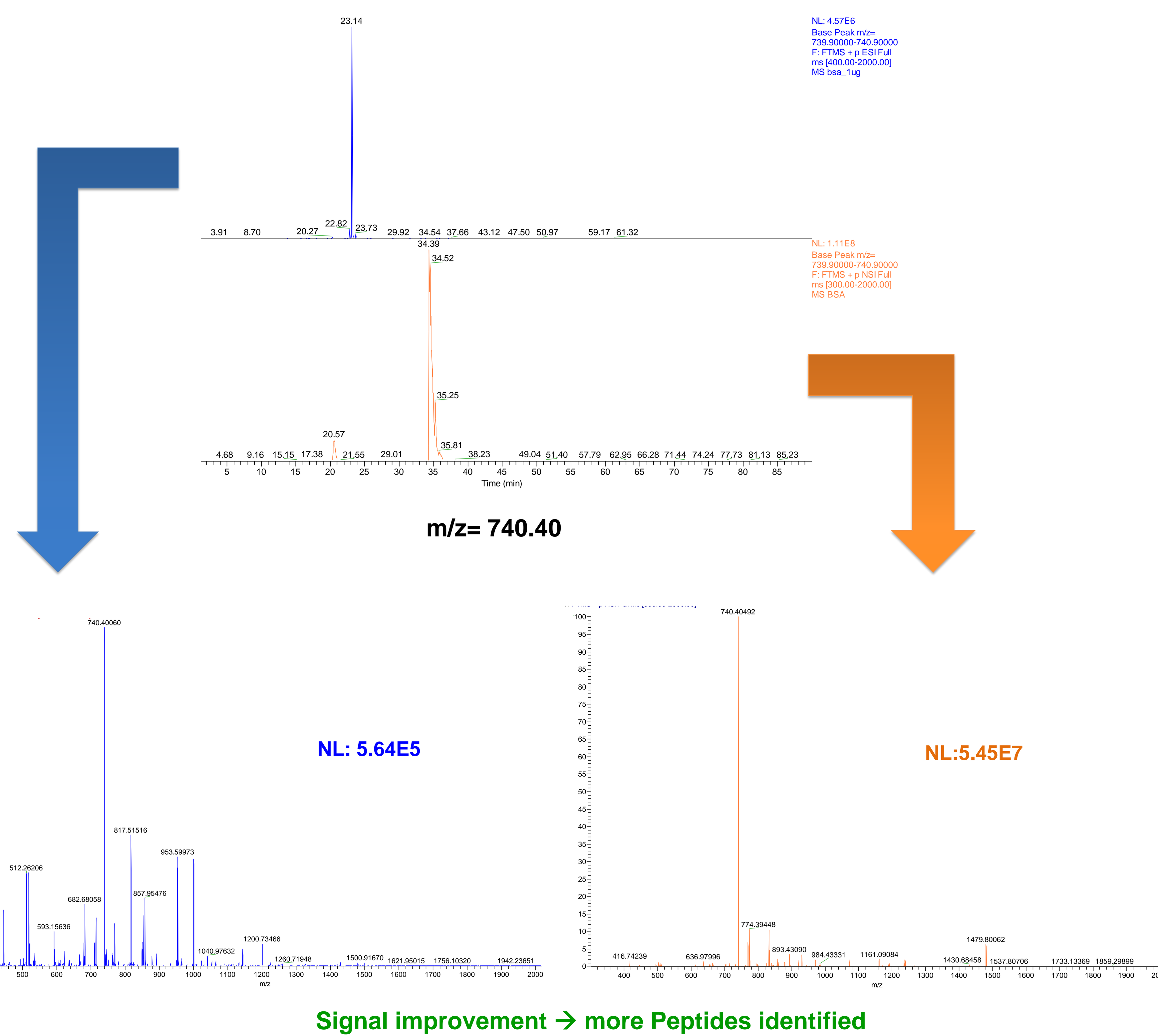
Thanks to Comissão de Coordenação e Desenvolvimento Regional do Norte for funding this project (ref NORTE-07-0162-FEDER-000048) through the program ON - Programa Operacional Regional do Norte (QCAIII). Thanks UNICAM –Sistemas Analíticos for all support.

RESULTS & DISCUSSION

Fig 1- LTQ Orbitrap XL™ hydrid mass spectrometer with Nano-EASY-Spray™ ion source.

Fig 2- LTQ Orbitrap XL™ hydrid mass spectrometer with electrospray ion source.





CONCLUSION

Orbitrap technology enables accurate mass, increases confidence of identification and gives access to qualitatively different information.

Using Dionex Ultimate 3000 RSLCnano-LC system with Nano-EASY-Spray™ ion source over the HPLC Accela Pump 600 *normal* electrospray ion source, allowed the increase of signal intensity and improvement of peptide identification.

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