

QUANTITATIVE PROTEOMIC ANALYSIS BY ITRAQ ON A LINEAR ION TRAP MASS SPECTROMETER

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One main objective of proteomic research is the systematic identification and characterization of proteins expressed in a biological system. The goal has recently extended to comparative and quantitative studies, thanks to recent advances in chromatography, mass spectrometry and bioinformatics. For this purpose there are an increasing number of approaches to quantify expression profiles from complex protein mixtures. The two most commonly used methods rely either on gel-based (*e.g.* DIGE) or on a chromatographic separation of proteins and/or peptides followed by mass spectroscopy (*e.g.* SILAC, iCAT, ^{18}O , iTRAQ).

Here, we describe an optimised methodology for the application of isobaric tags for relative and absolute quantitation (iTRAQ) and tandem mass spectrometry to obtain relative quantitative data from peptides derived from tryptic digestions of human plasma proteins previously depleted by using a multiaffinity removal system (MARS). The combination of the affinity chromatography for the elimination of the major abundant proteins of the plasma with the use of bidimensional liquid chromatography –using the approach of cation exchange, sample enrichment, reversed phase chromatography and microspray linear ion trap mass spectrometry¹– turns to be a powerful tool for the analysis of that kind of samples. The introduction of the pulsed quadrupole dissociation (PQD) mode has made analysis of iTRAQ reagent labelled samples possible². We developed software to extract and analyse quantitative data.

The method proposed here has been validated by the analysis of a tryptic digestion of BSA and casein standards. Optimised methodology is currently being applied in a multicentre study for the searching of cancer biomarkers in human plasma samples.

1. Mayya, V.; Rezaul, K.; Cong, Y.S.; Han, D.; *Mol. Cell. Proteomics*, **2005**, 4(2), 214-223.
2. Schwartz, J.C.; Syka, J.E.P.; Quarmby, S.P. *The 53rd ASMS Conference on Mass Spectrometry and Allied Topics*, San Antonio, TX, June 5-9, **2005**.