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# Biomedical Research Using Mass Spectrometry

**Abolgasam Jouban***
Pharmaceutical Analysis Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz

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**ABSTRACT**

As an alternative to array-based, gel-based, stable isotope tag or label-based methodologies, label-free mass spectrometric protein quantification methods have been developed to overcome the difficulties associated with differential expression proteomics. In this article, we concentrate on the problems with label-free techniques that use quantification based on peptide ion peak area measurements. Chromatographic alignment, peptide qualifying for quantification, and normalisation are some of these problems. In order to solve these problems, we present a number of methods that are combined in a recently created label-free quantitative mass spectrometry platform. These methods allow for thorough, precise, and repeatable protein quantitation in highly complex protein mixtures from experiments with numerous sample groups. We give a number of scenarios where the platform was successfully used to evaluate diverse protein expression or abundance in body fluids, in vitro Nano toxicity models, tissue proteomics in genetic knock-in mice, and cell membrane proteomics as illustrations of the usefulness of this approach.

**Keywords:** Biomedical Research; Mass spectrometry; Two-dimensional electrophoretic; Quantification

**Address for correspondence:**

Abolgasam Jouban,
Pharmaceutical Analysis Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
E-mail: abojouban@gmail.com

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## INTRODUCTION

The hardest part of proteomics technology is protein quantification for differential expression analysis or expression profiling. Typically, array-based, two-dimensional electrophoretic (2-DE)-based, or mass spectrometry-based techniques are used to do this task. MS-based approaches are typically referred to as "bottom-up" rather than "top-down," as the top-down approach has not yet realised its full potential. Complex protein mixtures are enzymatically digested in bottom-up quantitative methods, and the peptides from each protein are separated using liquid chromatography (LC) and detected using mass spectrometry (MS). Protein quantification is then finished at the peptide level, and the combined results are used to determine a summed value for the protein from which they originate [1]. Stable isotope labelling techniques were created early on in the development of quantitative MS-based proteomic technologies. This assumption has led to the emergence of numerous innovative label-based techniques. All of these, however, have a number of drawbacks, including (i) the need for additional sample processing steps in the experimental workflow, (ii) the high cost of the labelling reagents, (iii) inconsistent labelling efficiency, and (iv) the challenge of analysing low-abundance peptides in multiple samples, particularly when several experimental groups are being investigated [2].

We are overjoyed and thrilled that our special edition has been released. Modern mass spectrometry (MS) techniques have made enormous strides, and MS is now one of the fundamental analytical tools for biological and biomedical research. Drugs, metabolites, and biomolecules (proteins, peptides, oligosaccharides, lipids, DNA, and RNA) can all be quickly and accurately analysed using mass spectrometry. We have included 12 original research pieces and 2 review articles in this special issue. According to a phosphoproteome investigation of gossypol-induced death in ovarian cancer cell line, MS has been effectively used to do high-throughput proteome-wide analyses of proteins, protein-protein interactions, and protein posttranslational modifications (PTMs) in cells, tissues, or organs [3].

In their research, 3030 phosphoproteins were broken down into approximately 9750 phosphopeptides. Using a 2DGE-based Western blotting and MS/MS approach, they discovered that gossypol increased the expression of LATS1, which phosphorylates YAP1. They also reported the use of SILAC-based quantitative proteome analysis in myeloma cells and identified 24 glioblastoma tyrosine-phosphorylated proteins. They discovered that gossypol treatment caused cell necrosis and ROS generation. According to proteomics, gossypol caused changes in the

expression levels of 585 proteins, including the up- and down-regulation of factors related to death and DNA repair. In a distinct quantitative proteomic study, the differential protein expression in receptor activator of nuclear factor-B ligand-induced osteoclasts (RANKL-induced osteoclasts) in the presence and absence of oestrogen was thoroughly compared using a tandem mass tag (TMT) labelling technique [4].

We have also seen a rapid rise in metabolomics over the past few years, driven by MS analysis. The widespread use of MS has undoubtedly contributed to and will continue to aid development in our understanding of the molecular pathways that underlie biological processes. The application of MS to clinical molecular diagnostics is likely to happen soon. MS-based proteomics and metabolomics are playing a significant role in the finding of biomarkers for numerous human diseases and therapies. Presented a review on recent advancements in MS-based quantitative proteomics techniques, high-density lipoprotein (HDL) proteomics, and lipoprotein modification in biomarker discovery for atherosclerotic vascular disease additionally, the group investigated potential peripartum markers of infectious-inflammatory complications in preterm birth [5].

The RBP4 protein may serve as a biomarker for ovarian cancer, according to their proteomic findings. The RBP4 ELISA and immunohistochemical studies supported the proteomic findings. The work on the identification of serum peptide markers for gestational diabetes mellitus (GDM) was presented by T. Ai's team. MS is frequently used to conduct pharmacokinetics investigations of a drug's impact on various biological systems. In the study, pharmacokinetic studies of a synthetic drug for the treatment of spinal cord injury, M. Sánchez-Sierra et al. described MS. presented a comparative study on Nell-1 hypermethylation levels between tumour, Para carcinoma, and normal tissues from gastric cancer patients [6]. They also discussed an MS application on analysis of DNA methylation and/or hydroxymethylation. They described an LC-MS/MS method to quantify and compare simultaneously global methylation and hydroxymethylation in human DNA of different tissues. They discovered a substantial difference between normal tissue and gastric cancer or Para carcinoma in terms of DNA methylation state. H. Wang's group also contributed to this special issue with their description of the effective use of LC-MS for the quick distinction of -lactam and ring-opened -lactam impurities in cefixime, cefdinir, and cefaclor medicines [7].

## DISCUSSION

Under favourable ionisation circumstances, the electrospray ionisation of CrEL resulted in a large number of molecular ions. There were too many molecule ions present in the spectra, making it challenging to identify the one of interest. In the MRM method of analysis, the majority of the molecular ions failed to create any identifiable fragment ions. So, tuning solution was fed via an LC column into a mass spectrometer in order to comprehend the molecular ions that relate to the hydrophilic and hydrophobic sections. The developed 30 min LC gradient produced clear separation. While hydrophilic oligomers corresponding to PEG oligomers generated identical daughter ions with m/z 89.10 fragmentation pattern of one oligomer m/z 960.20, other oligomers also shared similar fragmentation pattern, hydrophilic oligomers corresponding to glycerol polyoxyethylene did not produce any distinct daughter ions [8].

Ammonium adducts were the molecular ions of PEG oligomers that were found. The calibration curve was constructed by adding the analyte peak areas of the four oligomers to determine the plasma concentrations of CrEL-PEG as a whole. Different daughter ions with m/z 307.10 were produced by hydrophobic oligomers of glycerol polyoxyethylene ricinoleate with molecular ions at 844, 888, 932, and 976. Despite having separate fragment ions, the hydrophobic component's determination was abandoned since rat plasma tests revealed it to be extremely unstable. Since none of the oligomers produced different fragment ions, the hydrophilic glycerol polyoxyethylene component was not included for analysis. PPG was electrosprayed with ions, which resulted in numerous molecular ions at m/z 906.70 and a potent fragment at 117.10 amu. For the analysis of CrEL-PEG in plasma, a 3.5 min general gradient LC method (Time (min)/%B = 0.01/5, 1.50/95, 2.50/95, 2.60/5, and 3.50/5) was developed using the C18 column and the LC-MS/MS technique [9].

For better peak shape and to address the response saturation seen at higher calibration standards for CrEL-PEG, a variety of organic modifiers including acetonitrile, methanol, and acetone were investigated. At higher calibration standards, all examined organic modifiers had poor linearity and response saturation. But when THF was added to acetonitrile, the linearity improved and response saturation was not observed. THF is the solvent of choice for the analysis of many polymers, but because it is a highly flammable solvent, larger concentrations of THF cannot be utilised in LC-MS/MS. Therefore, 20% THF in acetonitrile was used as the final mobile phase condition [10].

## CONCLUSIONS

For quality assurance and research in the realm of viticulture and oenology, mass spectrometry is crucial. These techniques are more suited for studying the structures of polyphenols and anthocyanins in grape extracts and for the research of structures connected to the colour change of red wines due to the soft ionisation conditions of LC/MS and the minimal sample purification typically required. The high-MW chemicals found in grapes, such as procyanidins, proanthocyanidins, prodelphinidins, and tannins, can also be characterised using these techniques. The significant number of publications that have been published in the literature over the past several years further supports the crucial function that LC/MS plays in the structural analysis of polyphenols.

The wide range of chemicals found in grape and wine can be characterised very effectively by the complementary application of various MS techniques. For instance, the

use of LC/MS, MALDI-TOF, and MS/MS techniques allowed the characterization of procyanidin oligomers up to dodecamers; the coupling of LC/MS with MS/MS techniques is very effective, especially for the characterization of glycoside compounds; and GC/MS and LC/MS analyses allow the characterization of hundreds of volatile and nonvolatile compounds, providing practically the entire grape metabolome. As these metabolomic methodologies are developed further, they will offer efficient methods for identifying a large number of significant chemicals in grapes with few analyses and little sample preparation, offering helpful details on the substances involved in cell and tissue metabolisms.

## ACKNOWLEDGMENT

None

## CONFLICT OF INTEREST

None

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