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Clinical peptide and protein quantification by mass spectrometry (MS)

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ABSTRACT

MS quantitation of peptides/proteins has been slow to be adopted by clinical laboratories. The reasons are: (i) lesser perceived need for assay improvement of protein/peptide immunometric immunoassays (IAs) compared to competitive IAs, (ii) increased demands peptides/proteins place on mass accuracy/resolution, (iii) limitations of current instrumentation, (iv) stickiness of peptides/proteins, (v) large number of serum peptides/proteins and their broad range of concentrations.

However, MS can (i) facilitate assay harmonization, (ii) identify clinically relevant protein-isoforms, (iii) solve the antibody interference and "hooking" artefacts of immunometric IAs, and (iv) facilitate biomarker verification/validation.

Consequently, the last 10–15 years have seen steady growth in peptide/protein MS assay-development. Common protocols are emerging; most can be performed on triple quadrupole instruments. Affinity enrichment, with antibodies against analyte or proteotypic peptides, is commonly used. Assay performance characteristics are similar to IAs.

The future will see standardization of assay validation/quality-control requirements and increasing use of multi-target assays.

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Abbreviations: 25-OH-D, 25-hydroxy Vitamin D; AA, African American; AIC, analyte immunocapture; aa, Amino acid(s); Angl, angiotensin I; TgAB, anti-thyroglobulin auto-antibody; AB, antibody; C, Caucasian; CV, coefficient of variation; ESI, Electrospray ionization; GH, growth hormone; HRAM, high resolution, accurate mass spectrometer; IA, immunoassay; MS, mass spectrometry; IGFBP3, IGF binding protein 3; IGF1, insulin-like growth factor 1; IS, internal standard; Lp(a), lipoprotein(a); LC-MS, liquid chromatography, mass spectrometry; LC-MS/MS, liquid chromatography, tandem mass spectrometry; m/z, mass over charge ratio; MS, mass spectrometry; MALDI-TOF, matrix assisted laser desorption ionization source coupled to a time of flight mass spectrometer; OT, orbital trap mass spectrometer; MW, molecular weight; O, other; PRA, plasma renin activity; PTH, parathyroid hormone; QQQ, triple quadrupole mass spectrometer; QOT, quadrupole orbital trap mass spectrometer; QTOF, quadrupole time of flight mass spectrometer; RIA, radio-immunoassay; SIM, selected ion measurements; SRM/MRM, selected/multiple reaction monitoring mode; SPE, solid phase extraction; S/P, serum or plasma; SISCAPA, stable isotope standards and capture by anti-peptide antibodies; Tg, thyroglobulin; TPCK, L-(tosylamido-2-phenyl) ethyl chloromethyl ketone; TOF, time of flight mass spectrometer; U, urine; VDBP, vitamin D binding protein.

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1. Introduction

During the last 20–30 years, mass spectrometry (MS) has migrated from the research realm into the clinical laboratory. MS is now routinely used in many clinical laboratories for quantification of a huge range of low molecular weight (MW) compounds (<1500 Da) in biological samples [1–5]. In the past, competitive immunoassays (IAs) were used for these analytes, because their small size precludes the use of sandwich-type IAs. Virtually all competitive IAs suffer from three key weaknesses: (i) poor harmonization between different assays for the same analyte, (ii) limited dynamic range (<2 orders of magnitude), and (iii) high susceptibility to cross reacting substances. These limitations are, to varying degrees, addressed by MS assays.

However, until recently, there have been few clinical quantitative MS assays for peptides or proteins. In this review we will (i) explore the reasons for this, (ii) explain why this has recently changed, (iii) illustrate the workflows used, and (iv) give some assay examples.

1.1. Reasons for the late adoption of clinical quantitative peptide/protein mass spectrometry

(i) Most of the clinically relevant peptide and protein targets are large enough to allow the use of immunometric IAs. While immunometric IAs share the harmonization problems of competitive IAs, they typically have a dynamic range of >2 orders of magnitude and exhibit much reduced cross reactivity. The advantages of a switch to MS are therefore less compelling for peptides/proteins.

(ii) Electrospray ionization (ESI), the most commonly used ionization method (Fig. 1), results in nearly all peptides and proteins carrying multiple charges [6,7]. The number of charges for globular proteins is related to their MW by a square root function[1]. In the mass range of 50–200 KDa this translates into ~10 to 35 charges per molecule [6,7]. However, even small peptides of 6–12 amino acids usually carry at least 2–3 charges; both N-terminus and C-terminus can be ionized, as can internal amino acids that are capable of carrying a charge under the given source conditions. Furthermore, several populations of ions with different charge states are usually simultaneously present (at least 5 or 6 for modestly large proteins). Each of these charge states has its own isotopic envelope, which is much wider and more complex than the isotopic envelope of low MW compounds. This all creates a very complex mass spectrum, placing high demands on mass accuracy and resolution of MS equipment, and on post analytical data processing. At the same time, the spread of the signal across many charge states complicates quantitation – which of the peaks of which charge state should be used, should peaks be summed, should isotopic peaks be ignored or deconvoluted, etc. – and often compromises detection sensitivity. Given the high demands on mass accuracy and resolution, high resolution, accurate mass (HRAM) instruments, such as Time of Flight (TOF) mass spectrometers (TOF, or Quadrupole-TOF [QTOF]), or Fourier transforming mass spectrometers (electrostatic orbital traps [OT], or QOT) are often better suited for these applications than the most commonly used clinical instruments, triple quadrupole (QQQ) mass filtering devices (Fig. 2). Unfortunately, HRAM instruments tend to have a narrower dynamic range and poorer detection sensitivity than QQQs [8–14]. Most current MS instruments have limited mass-over-charge (m/z) ranges. This impacts their ability to accurately identify and quantitate large proteins with good sensitivity, hence, the common use of enzymatic digestion before MS analysis. For QQQ instrumentation, the upper mass range limit is typically 2000–4000 m/z. Most QTOF, QOT and OT mass spectrometers achieve 5000–30000 m/z, depending on model and configuration [8,10,11,13,14]. However, if they contain quadrupoles their usable m/z range might be lower than that. Quadrupole mass filters select ions based on their trajectories in an electrical field consisting of a constant voltage along the axis of the ion trajectory (z) and modulated variable voltages that are applied in the x and y directions. Based on the limitations of current electronics and instrument design-choices, accurate m/z selection by this method is typically limited to a maximum of 3000–4000 [9,10]. Above this, efficiency of ion detection falls sharply for any device that incorporates a quadrupole and uses an ion impact detector, i.e. QQQ and QTOF instruments, as increasing numbers of unwanted ions are no longer filtered out and compete with target ions for detection. QOT instruments should be less affected, because their detector works continuously; however, the OT will eventually be overfilled,

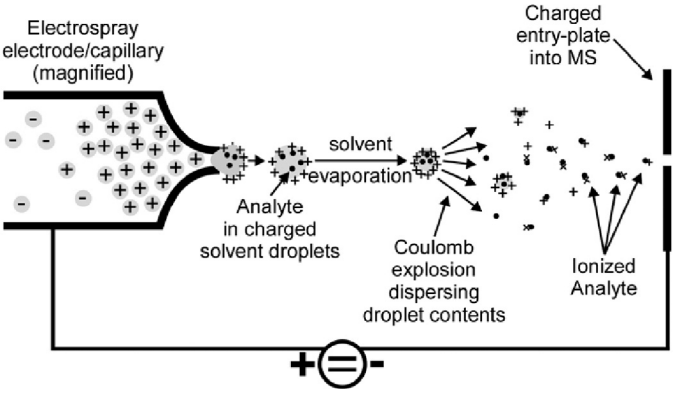

Fig. 1. Schematic depiction of an electrospray ionization (ESI) source. ESI is the most frequently used ionization method in mass spectrometry of peptides and proteins. It results almost always in multiply charged ions, with the number of charges related to the peptide's/protein's molecular weight (see main text).

[1] $Z_R = 4\left(\frac{\pi\gamma\varepsilon_0}{\rho e^2 N_A}\right)^{1/2} \times M^{1/2} = 0.078\,M^{1/2}$, Z_R : charge at Rayleigh limit (just before Coulomb explosion), γ: surface tension of solvent, ε_0 : permetivity of surrounding medium, ρ : density of water, N_A : Avogadro's number, M : molecular weight of protein.

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