Analysis of biopharmaceutical proteins in biological matrices by LC-MS/MS II. LC-MS/MS analysis

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The first section of Part II describes the current strategies for mass spectrometric (MS) detection in the selected reaction monitoring (SRM) mode which has become the method of choice to quantify peptides. We then discuss the selection of signature peptides, SRM transitions and labeled internal-standard peptides to obtain the best assay selectivity. We also present improved assay selectivity on triple-quadrupole linear ion trap using MS^3 and differential mobility MS. We dedicate the final section to alternative approaches based on high-resolution data-independent acquisition.

Keywords: Biopharmaceuticals, Peptide; Protein, Quantitative analysis, Selected reaction monitoring (SRM), Assay selectivity; Biological matrix; Signature peptide; High resolution mass spectrometry, Ion mobility, MSn

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1. Introduction

Biopharmaceuticals represent a significant portion of the pharmaceuticals market. In 2009, sales of biologics totaled $99 billion, 12% of the total market, including the $38 billion of the monoclonal-antibody (mAb)-related product (5% of the total market) [1]. The current gold standards for protein quantification are ligand-binding assays [e.g., enzyme-linked immunosorbent assay (ELISA)]. This analytical method is based on immuno-affinity and offers high sensitivity, but the dynamic range is often limited to one to two orders of magnitude. However, commercial assays are available for a limited number of proteins and the development of new antibodies is a time-consuming, costly process. ELISA may also suffer from selectivity issues and results can be significantly altered depending on the kit manufacturer [2]. Also, when multiplexed assays are designed, potential interferences from cross reactivity must be assessed.

The alternative of protein quantification by LC-SRM/MS was recently applied to quantify therapeutic proteins (e.g., mAbs and recombinant proteins) [3–8]. The methodology took inspiration from the well-established quantification of small molecules by LC-SRM/MS in the field of bioanalysis in the pharmaceutical industry, anti-doping, and forensics [9]. The assays tend to monitor only one protein in a large number of samples, so development and optimization of the analytical method, from sample preparation to MS-detection method, is focused on the development of a specific analytical method dedicated to the quantification of a particular target. Practically, quantification is based on the construction of calibration curves and needs to fulfill validation criteria {e.g., those of the Food and Drug Administration (FDA) guidelines [10,11]}. The use of isotopically-labeled synthetic peptides or proteins as internal standards (ISs) is necessary to balance the fluctuations inherent to the sample-preparation and mass-spectrometer response.

The most predominant biological matrices for biopharmaceuticals are plasma and serum, which contain, in addition to the abundant proteins, informative proteins (e.g., hormones, cytokines, and proteins leaked from tissues). The protein concentrations are spread over 10 orders of magnitude (albumin represents 55% of the total plasmatic proteins) [12,13], so the analytical dynamic range represents a real challenge.

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This article is Part II of the review on detection and quantification of therapeutic proteins in complex biological matrices. While Part I [14] focuses on various sample-preparation steps, Part II addresses mass spectrometric (MS) and chromatographic aspects, in particular the selection of signature peptides, the selectivity of the selected reaction monitoring (SRM) mode and applications of high-resolution MS (HRMS).

2. SRM detection and signature-peptide selection

In bioanalysis, proteins are not frequently quantified in their intact form (top-down) by LC-MS(/MS) because they display a broad range of size and chemical properties that require specific chromatographic and MS ionization conditions to be measured. In the bottom-up approach, the specific peptides resulting from an enzymatic digestion are analyzed as the surrogates of the targeted proteins. For quantitative purposes, the SRM mode of the triple quadrupole (QqQ), introduced by Enke and Yost in 1978 [15], has become the main asset of the QqQ design. Practically, the analysis by the SRM mode is provided by the selection at unit mass of a precursor ion on quadrupole Q1 and a fragment ion, produced by collision induced dissociation (CID) in the collision cell, on quadrupole Q3 of the mass spectrometer. While, for low-molecular-weight compounds, a single Q1-Q3 transition is used for quantification and a second one for confirmatory analysis, several transitions are generally recorded in the case of peptides. Another key aspect is that the quantification is performed at a peptide level and extrapolated to the protein. Selectivity is therefore becoming a key issue regarding the selection of the peptides and the analytical measurement. As illustrated in Fig. 1, several factors (e.g., selection of the SRM transitions, sample preparation or chromatography) can affect the analytical read-out. In peptides analysis, further specificity comes because these molecules are usually ionized in electrospray under a multi-charged form

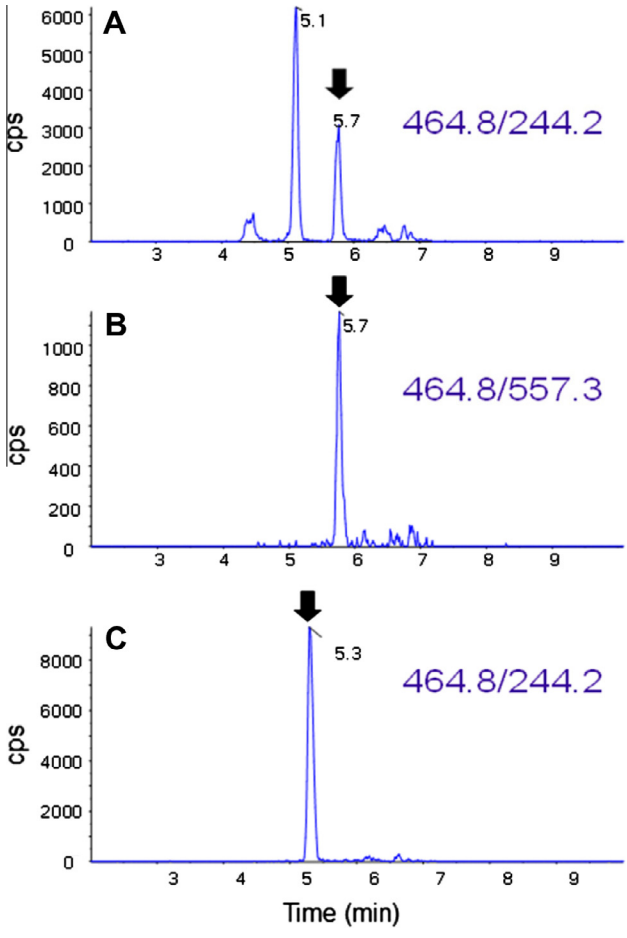

Figure 1. The selectivity of an LC-SRM/MS method depends on the monitored fragment, the chromatographic separation and the sample preparation. (A) Plasma treated by selective precipitation (45% v/v, CH_3CN) before digestion. SRM transition *m/z* 464.8 → *m/z* 244.2. (B) Plasma treated by selective precipitation (45% v/v, CH_3CN) before digestion. SRM transition *m/z* 464.8 → *m/z 557*.3. (C) Plasma treated by selective precipitation (50% v/v, CH_3CN) before digestion. SRM transition *m/z* 464.8 → *m/z* 244.2.