JOURNAL OF PROTEOMICS XX (2013) XXX–XXX

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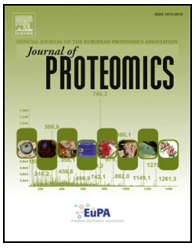

Review

Isotope dilution mass spectrometry for absolute quantification in proteomics: Concepts and strategies

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ARTICLE INFO

Article history:
Received 31 October 2013
Accepted 1 November 2013

Keywords:
Internal standard
Isotope dilution mass spectrometry
Mass spectrometry
Quantitative proteomics
Absolute protein quantification
Quality control

ABSTRACT

Isotope dilution mass spectrometry is a reference technique for quantitative analysis, given that it combines the sensitivity and selectivity of MS instruments with the precision and accuracy associated with the use of internal standards. Isotope-labeled proteins are the optimal internal standards for quantitative proteomics as they closely mimic the behavior of their natural counterparts during the analytical process. A major complication of isotope dilution mass spectrometry proteomics is the technical difficulty of obtaining these internal standards, especially in studies where a high number of proteins have to be quantified simultaneously. In this paper, we review some of the characteristics of the isotope dilution mass spectrometry approach, its benefits in terms of reliability and quality control in targeted proteomic analysis and the different strategies developed for its application in proteomics.

Contents

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1874-3919/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.jprot.2013.11.004

1. Introduction

Quality control procedures such as those related to method validation and data traceability are well implemented in clinical, environmental and drug development laboratories [1–11]. With the development of new protein biomarkers for approval by regulatory agencies, there is the need to develop analytical methods to provide protein absolute quantitative data of controlled accuracy and precision [12–14]. Isotope dilution mass spectrometry (IDMS) is the optimum analytical technique to provide reliable MS-derived absolute quantitative data [15–17]. IDMS involves the addition to the sample of an isotopically labeled compound, the internal standard (IS), followed by the simultaneous determination of both the analyte and IS by mass spectrometry. IS measurements are used to neutralize changes in the analytical performance over time and between laboratories affecting the analyte values. Typically, quantification-oriented LC-MS methods use selected MRM (multiple-reaction monitoring) transitions to obtain optimum selectivity, sensitivity and precision in the detection and quantification of analytes and internal standards [12,18].

In the past, quantification of larger proteins using IDMS has been limited by a combination of technical problems that have complicated the retrieval of accurate and reproducible absolute quantitative concentration values. The technological achievements of the last decade have solved many of these limitations, and it is currently feasible to use IDMS for the absolute quantification of a number of proteins in biological samples [19–22]. Problems still exist, however, for its application on proteome wide experiments, namely the availability of standards for each of the proteins analyzed and the confinement of the approach to targeted experiments. In addition, the process of synthesizing and purifying isotopically labeled proteins for their use as IS can be expensive both in terms of economic costs and of method development time [13,15,23]. As a consequence, along the years, several alternative strategies have been developed for protein quantification. These approaches include methods which have been reported to produce absolute quantification data of reasonably good precision without the use of internal standard normalization [24] as well as other well-known approaches using isotopic labelling (e.g. ICAT, iTRAQ, TMT, SILAC) which have been extensively used over the last 15 years to quantify relative differences between a limited number of samples [25–29]. For example, Zhang et al. [24] performed a reevaluation of the raw data from an inter-calibration exercise [30] and calculated that the variability without IS still remained around the acceptable limit for a quantitative method (CV 20–30%, the CV of the original study was ca. 10%). The authors concluded that absolute quantification without IS provided cost-effective alternatives to IDMS for many multiplexed MRM LC-MS applications [24]. Along the same line, a growing number of reports are presenting label-free approaches as viable alternatives to isotopic labeling for relative quantitation [28,31–39].

Quantification without IS has however important drawbacks. Any LC-MS-based analytical method contains a number of critical points that may lead to biased results, such as LC column deterioration, changes in MS response factor over time, low analyte recovery, matrix effects, and human errors [12,30]. Without IS, the number of failed runs related to unpredictable events is liable to increase dramatically, depending on the long-term stability of the analytical setup [42]. These problems are well-recognized in most fields that have been using MS for quantitative purposes for decades [1,8,42–47]. The absence of IS must therefore be compensated by implementing procedures to minimize possible sources of errors, with consequent costs in terms of time and performance. Even if these problems are conveniently considered and neutralized, possible matrix effects can produce biases (systematic or random) that cannot be detected nor compensated without IS [12,48,49].

Thus, in comparison to other approaches, IDMS methods hold the potential to provide absolute quantification data of the highest precision and of controlled accuracy. The characteristics of IDMS quantification have made this approach the basis for many Definitive and Reference methods in Clinical Chemistry. The correct application of the IDMS implies that quantification results will be traceable to a common (i.e. international) reference standard, a condition which allows the comparison of the results from an unlimited number of experiments as well as inter-laboratory/inter-method comparison [40]. In this respect, the implementation of quality control procedures that ensure the accuracy and precision of the quantitative data is a basic requirement for successful data comparison [41].

2. General concepts in quantitative mass spectrometry

The comparison of different datasets cannot be reliably performed unless there is the certainty that varying laboratory conditions (different analysis date, laboratory, instrumental setup and reagents) as well as variability in the sample matrix have not introduced biases into the quantitative data [5,41,50]. To achieve this goal, both calibration standards and analytical procedures are required to be technically appropriate and validated [41,51] (Fig. 1).

The calibration standards are samples of controlled composition that allow the transformation of the intensity

Please cite this article as: Villanueva J, et al, Isotope dilution mass spectrometry for absolute quantification in proteomics: Concepts and strategies, J Prot (2013), http://dx.doi.org/10.1016/j.jprot.2013.11.004