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SOLUTIONS FOR BIOANALYSIS

A universal approach for individual identification and quantities assessment of Host Cell Proteins with LC-MS

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INTRODUCTION

Host cell proteins (HCPs) from biologics production is one of the growing concerns for biopharma companies regarding their putative immunogenicity in human beings treated with biologics. Currently, HCPs are typically quantitated using biochemical or immunological techniques (ELISA assays). However, the development of ELISA for newly detected proteins is time consuming and requires specific reagents. These drawbacks limit the number of quantified & identified specific HCPs. Mass spectrometry (MS) has the potential to overcome these limitations. The ambition of this development was to develop an universal MS-based HCPs quantitation kit coupled to label-free (LF) approach, to simultaneously identify and accurately asses individual HCPs quantities in complex products in a single MS run.

STRATEGY

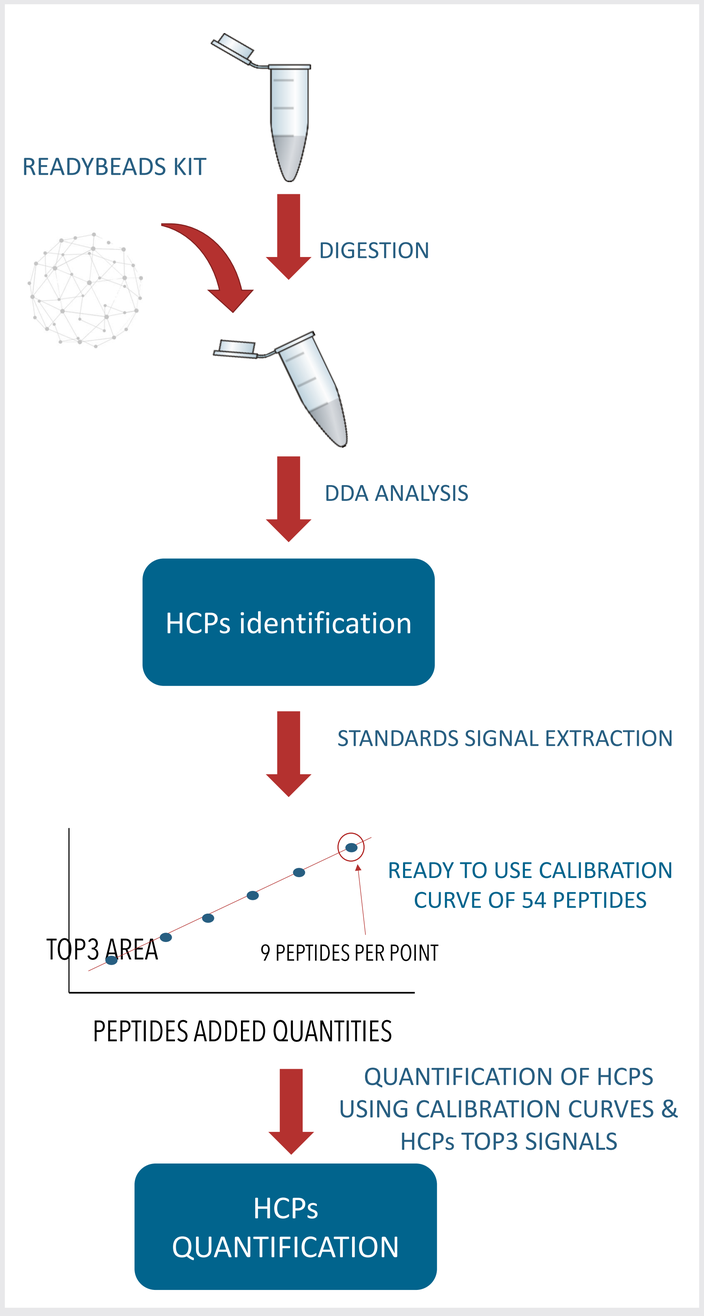

Figure 1: analysis pipeline with READYBEADS HCP kit.

A Label-Free approach based on the top 3 principle (Silva et al., 2006) was developed to have a global strategy implementable to any biologic sample. The first step was to generate a reference calibration curve for quantification. As standards, 54 different peptides, coming from 18 proteins were selected based on their sequences and molecular weights (Trauchessec *et al.*, 2014). The peptide mixture was formulated into a large dynamic range. These peptides were coated on the READYBEADS™ technology. This technology allows a fast and reproducible preparation of standard solutions and particularly avoids storage, weighing and stability issues. Figure 1 shows the use of READYBEADS™ Kit to generate a calibration curve and the analytical workflow to identify and quantify HCPs in samples.

EXPERIMENTAL PROCEDURE

To assess READYBEADS™ kit performances, 2 ways were explored, the accuracy and reproducibility. So that, 2 batches of READYBEADS™ Kit were independently produced. READYBEADS™ Kits were added into 2 replicates of a same therapeutic sample and calibration curves obtained after a DDA analysis, allowing both identification & quantification of HCPs with 2 different batches. Then, to control the accuracy of quantification, BSA was added at 3 different concentrations in 3 different samples, since all samples were analyzed in technical triplicates.

CALIBRATION CURVES REPRODUCIBILITY

Results obtained for calibration curves (figure 2) demonstrated highly reproducible slopes intra & inter-batches of READYBEADS™ kit.

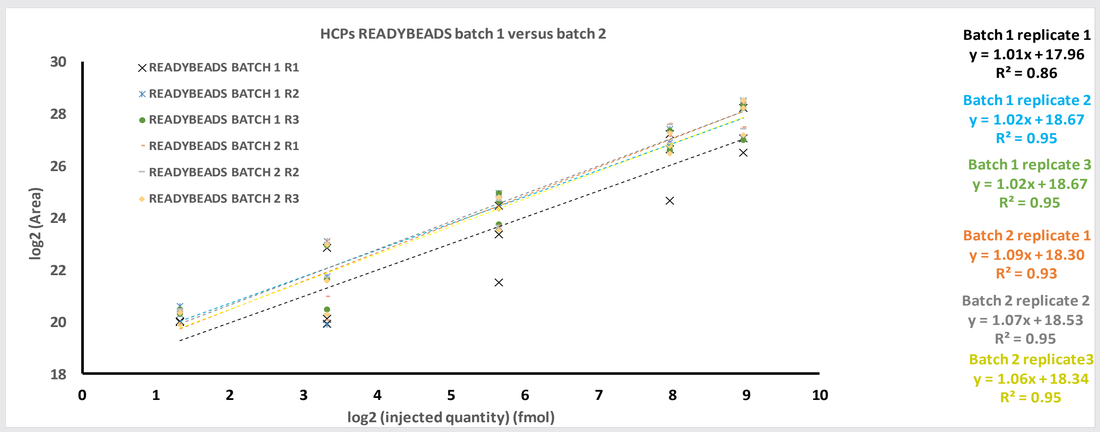

Figure 2: calibration curves results for 6 different READYBEADS™ from 2 batches.

QUANTIFICATION REPRODUCIBILITY

600 HCPs were quantified into samples, with 2 batches of READYBEADS™ kit. For each HCP, the ratio of concentration obtained with the two batches was calculated. The Gaussian profile centered on 1 (figure 3) demonstrates a good reproducibility of HCPs quantification.

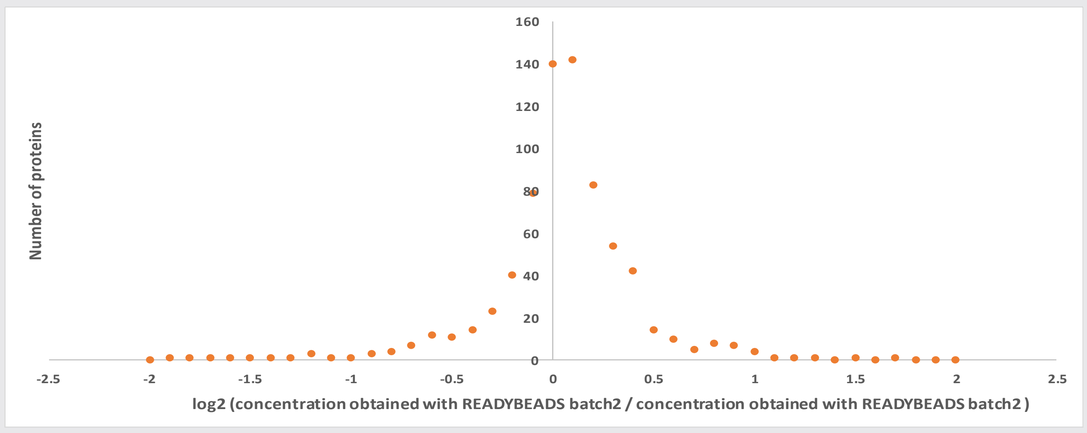

Figure 3: ratio of concentration obtained with READYBEADS™ kit 1 / READYBEADS™ kit 2.

QUANTIFICATION ACCURACY

BSA from NIST was spiked into samples (table 1). A good correlation between the theoretical and calculated concentration was found (<33 % of

	Added quantity	CV	Calculated quantity average 3 replicates	Deviation from theoretical concentration
	µg/ml	%	µg/ml	%
BSA	0.21	7.8	0.18	-14%
	2.08	11	1.93	-7.0%
	8.32	18	11.0	33%

Table 1 : quantification results obtained for the benchmark of accuracy (BSA).

CONCLUSIONS

A newly developed HCP quantitation kit, coupled to Label-free LC-MS strategy was developed to identify and accurately assess the quantities of hundreds HCPs in a complex biologics products, in a single MS run. This kit can be universally used at different steps of down-steam process, since internal calibration curves can normalize whatever the matrix effects.

REFERENCES

Silva et al. , Mol Cell Prot, 2006 ; Trauchessec et al., Mol Cell Prot, 2014. Corresponding authors: anaquant@anaquant.com