Specific quantification of polyvalent formulated vaccines by multiplex LC-MS/MS analysis

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INTRODUCTION

Vaccines principle relies on pathogenic-based substances called antigens triggering immunological response, and production of memory cells. Today, pharma trends to develop multivalent vaccines to both decrease production cost and limit inconvenience for patients. Antigens are produced separately and quantified before to be mixed together during the formulation step. Final product presents a real analytical challenge, since classical tools such as immuno-affinity or LC-UV do not provide enough specificity to discriminate antigens with similar sequences.

However, antigens quantification, and batch-to-batch comparison of final product is crucial to ensure treatment safety and efficacy. Mass spectrometry coupled to liquid chromatography (LC-MS) recently appeared as an orthogonal analytical solution, in regards of its intrinsic specificity properties.

PROBLEMATIC AND OBJECTIVES

In this poster we developed LC-MS method to specifically quantify 4 antigens, with very similar sequences, in formulated vaccines during a single analysis. Results were correlated with values obtained individually before formulation on each antigen, with classical approach (figure1).

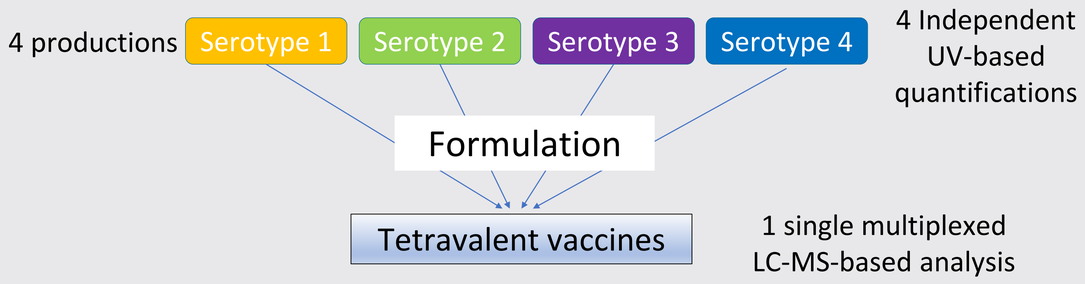

Figure 1: Classical vaccine characterization in Pharma industry and advantage of LC-MS/MS for final product control

ANALYTICAL APPROACH & CHALLENGES

The developed approach must deal with 2 technical challenges:

- Specifically identify and quantify 17 strains containing sequence homologies encompassed between 50 and 85%.
- Ensure reliable and reproducible quantification after formulation

LC-SRM (Selected Reaction Monitoring) analysis coupled to stable isotope dilution strategy (SID) appeared judicious to answer these requirements.

SRM targeted approach is considered as the golden one in regards to its intrinsic specificity properties. Isotope dilution relies on the addition of heavy peptide standards perfectly calibrated in samples. These latters allow back-calculating endogenous quantity of peptide of interest (Figure 2). To ensure internal standards stability and reproducibility, READYBEADS™ technology was used, which consists in adsorption of peptide standards onto hydro-soluble polymer. Finally the following analytical workflow was deployed (figure 3).

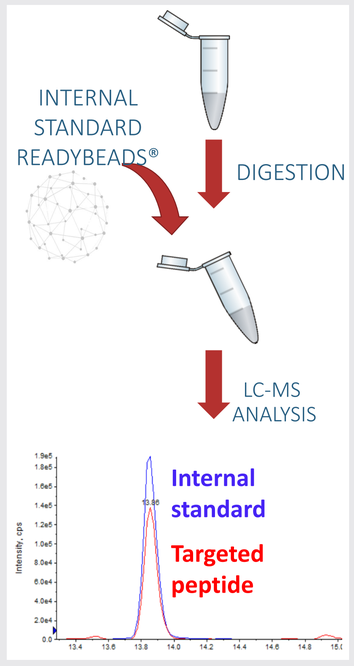

Figure 2: READYBEADS® used as internal standard.

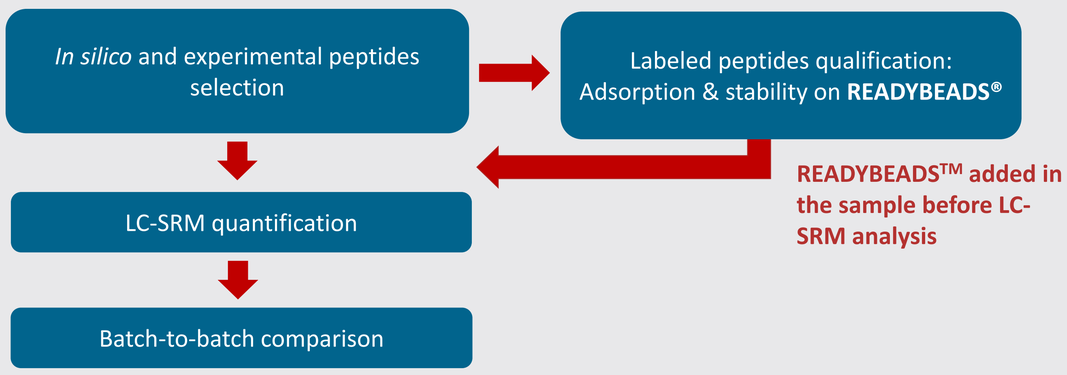

Figure 3: Analytical strategy for multiplexed & specific antigen quantification in formulated vaccines

INTERNAL STANDARDS STABILITY

Internal standards (heavy peptides) on READYBEADS™ were monitored and demonstrated stability over 7 months (Figure 4). READYBEADS™ allows over-passing stability and reproducibility issues encountered with SID strategy.

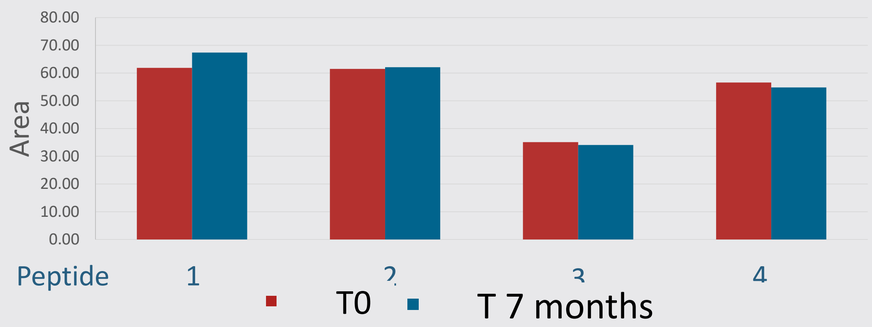

Figure 4: heavy peptides stability on READYBEADS™

BATCH-TO-BATCH COMPARISON IN FORMULATED VACCINES

7 tetravalent formulated vaccine batches containing different quantities of antigens were analyzed. Figure 5 demonstrates that results obtained in formulated vaccines with LC-SRM approach are in line with those obtained before formulation with classical approach. Thus, the developed method allows accurate, specific and multiplexed quantification of antigens in a single MS-based analysis.

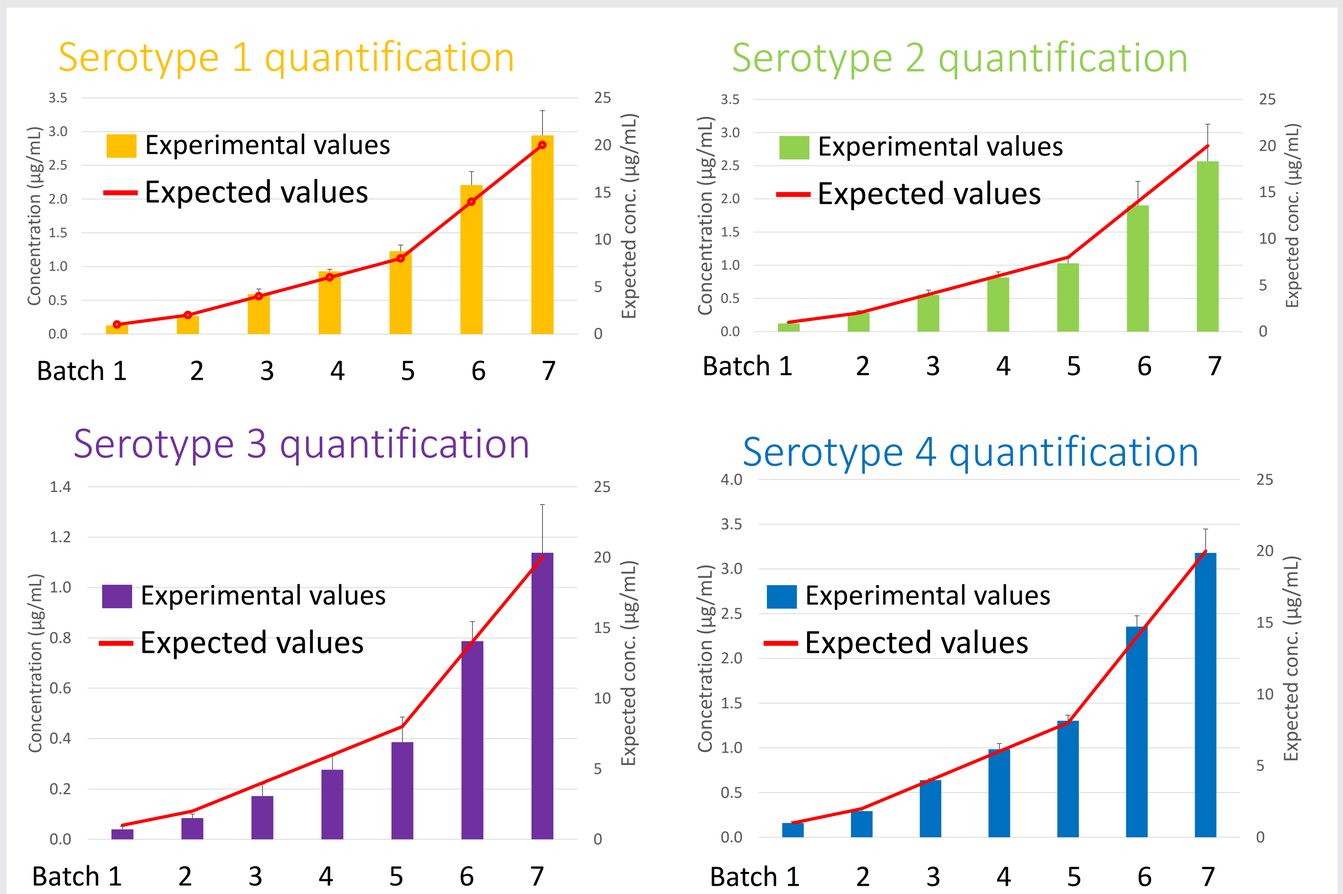

Figure 5: batch-to-batch comparison of antigens concentrations estimated in tetravalent vaccines, either with classical approach (4 different analysis before formulation), or with LC-SRM developed approach (single analysis after formulation). (n=6, 2 analytical sessions & 3 technical replicates).

CONCLUSION

These qualified internal standards, stable over at least 7 months, allowed batch-to-batch comparison of 7 tetravalent formulated vaccines. Results demonstrated a very good correlation between provided values, obtained independently before formulation, and measured concentrations in the final products. It was demonstrated that LC-SRM technology permits to specifically & simultaneously identify and quantify different serotypes in formulated batches. A specific LC-MS method coupled to READYBEADS™ for 17 strains is currently under development.