Specific quantification of antigens in polyvalent vaccines for batch-to-batch comparison by LC-MS

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INTRODUCTION

Vaccines contained pathogenic-based substances (virus or bacteria) called antigens. Inoculated to subjects under its inactive form (non-toxic), they generate an immune response permitting to be protected against the injected form. Today, the need is developing vaccines combining various diseases and various forms (strains) to improve vaccine accessibility, simplify the overloaded immunization schedules and decrease the health costs.

Analytical tool for vaccine characterization and batch-to-batch comparison during bio-manufacturing or after formulation is critical to ensure high quality treatment to human or animals.

Mass Spectrometry coupled to Liquid chromatography (LC-MS) is now recognized as a tool of choice for protein analysis in complex matrices, and here we developed an LC-MS method for the specific quantification of polyvalent vaccines.

CONTEXT AND NEED

The company Boehringer-Ingelheim develops bi, tri and tetravalent vaccines based on antigens from respectively 4 different strains (S1, S2, S3 and S4). The 4 strains are produced separately and mixed together during the formulation with the adjuvant. To confirm the correct formulation, there is a need to specifically detect and quantitate the 4 strains within the same formulated vaccine. This quantitative strategy will also be used for batch-to-batch comparison.

ISSUE

Some of the antigen sequences are really similar (from about 50 to 85% of homology), the analytical method needs to be specific enough, to differentiate the 4 strains.

Figure 1 presents the global workflow for specific strain analysis in formulated vaccines.

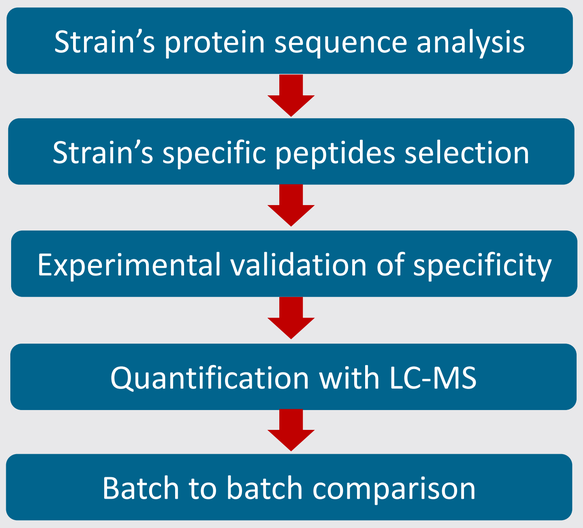

Figure 1: global workflow for specific antigen strain analysis in formulated vaccine

SPECIFIC DETECTION VALIDATION

To perform these analyses, LC-MS in *Selected Reaction Monitoring* (SRM) was selected. This mode is the method of choice for protein quantitation thanks to its unique potential for reliable quantification of low abundant analytes in complex mixtures. SRM analysis is based on unique peptides generated after enzymatic digestion of the protein mixture.

The first step of the analytical development is to check the specific detection for each strain. Figure 2 presents the specificity of each strain thanks to their respective unique signature peptides. The 4 peptides were monitored in each active principle before formulation and expected peptides were detected (signature peptide of S1 in S1 sample, signature peptide of S2 in S2 sample …). On the right hand side chromatogram, the specific detection of the 4 strains in a tetravalent vaccine after formulation is presented.

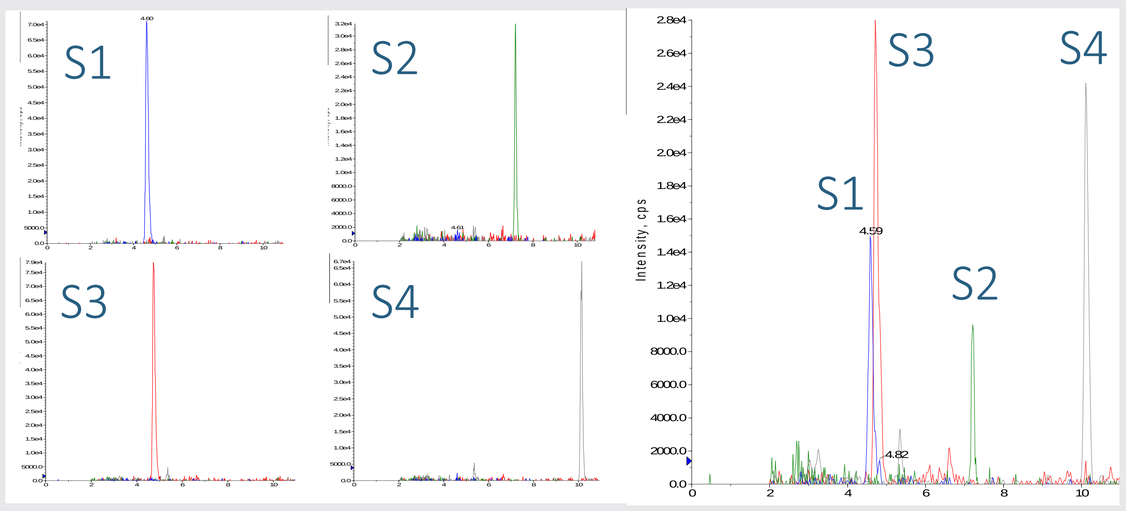

Figure 2: Detection of specific peptide strain signatures

LINEARITY VALIDATION

Detection linearity is important for monitoring antigen strains after formulation. Figure 2 presents the calibration curve of the detected signal for Strain 1 antigen over the dynamic range of interest.

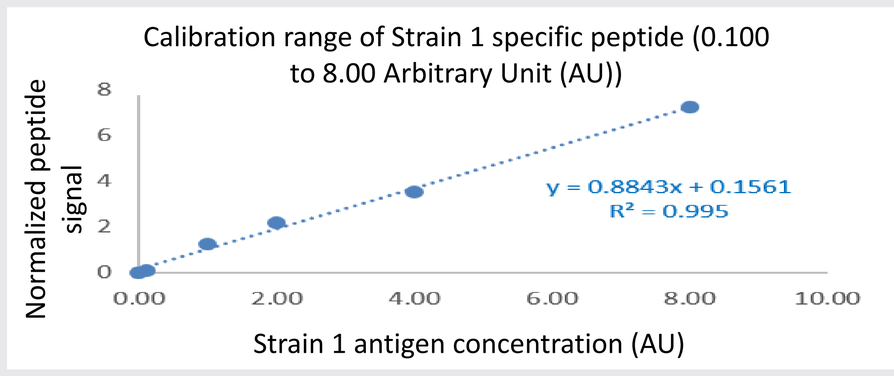

Figure 3: linear detection of Strain 1 antigen

BATCH-TO-BATCH COMPARISON

Since LC-MS has been demonstrated specific to differentiate the 4 strains over a broad enough dynamic range, the analytical method was used to compare different batches after formulation. Two different formulations were processed: Formulation A and B.

Figure 4 represents a batch-to-batch comparison of 6 different batches. Expected ratios are described above the pie chart and experimental ratio are shown within the pie parts. For most of the production batches (1, 2, 4 and 5) the analysis confirm the expected ratios. However, for batches 3 and 6, major differences are detected with unexpected ratios. Further investigations could be triggered to understand where the issue comes from: antigen production, vaccine formulation or analytical process.

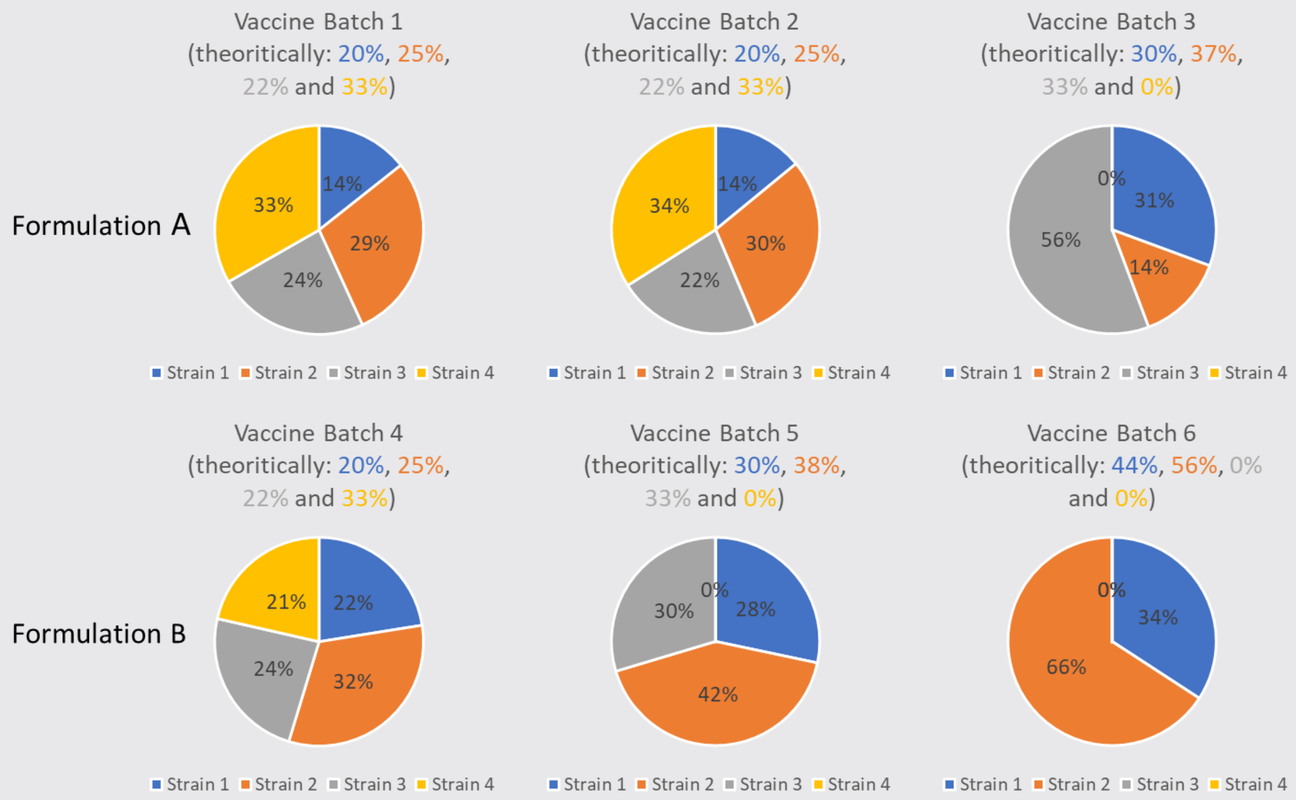

Figure 4: batch-to-batch comparison of different formulated batches.

CONCLUSION

This poster presents the use of LC-MS as a reliable quantification method for the characterization of multivalent vaccines thanks to their unique peptide signatures. The 4 different strains can be specifically detected and quantitate in final vaccine product. This analytical method was also used for batch-to-batch comparison and formulation validation in vaccine bio-production monitoring.