Mass spectrometry-based identification of allergen proteins involved in seafood-related allergic reactions

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Shellfish are one of the most common causes of food allergies and a major cause of food-induced anaphylaxis. The prevalence of seafood allergy is higher in populations residing in coastal geographic areas where seafood is an integral part of their diet. Sensitization and subsequent reactions occur most frequently upon ingestion. However, they can also occur because of skin contact. Shrimps are, among all, the most consumed type of seafood worldwide and for that it is important to identify and characterize all possible allergens. A bottom-up proteomics approach, LC-MS/MS coupled with Parallel Reaction Monitoring (PRM) technique, is used to acquire high-resolution full MS/MS spectra for each target allergen peptide. Total protein extracts from shrimp (*Penaeus monodon* and *Penaeus vannamei*) were isolated and processed through in-gel tryptic digestion of SDS-PAGE gel fractions or using PreOmics columns with or without fractionation. Resulting peptides were then collected and purified prior to LC-MS/MS analysis and the MS raw files were processed by the SEQUEST algorithm within the protein database for decapods (TaxID = 6683). In all shrimp samples it was possible to accurately identify our proteins of interest. Tropomyosin proteins specific for shrimp, prawns, lobster and crab were identified in our discovery workflow sharing a sequence identity between 89% and 100%. To support our findings, a PRM analysis was then performed looking for all shrimp unique tropomyosin peptides. A transition list for each peptide, from in silico digestion, is generated and analyzed within the Skyline open source software. It was possible to confirm the presence of the tropomyosin allergen and the results obtained suggest the reproducibility of this proteomics workflow, so as to be used not only in the identification of other important allergens in seafood-related allergic reactions but also of allergens involved in other types of allergic diseases.

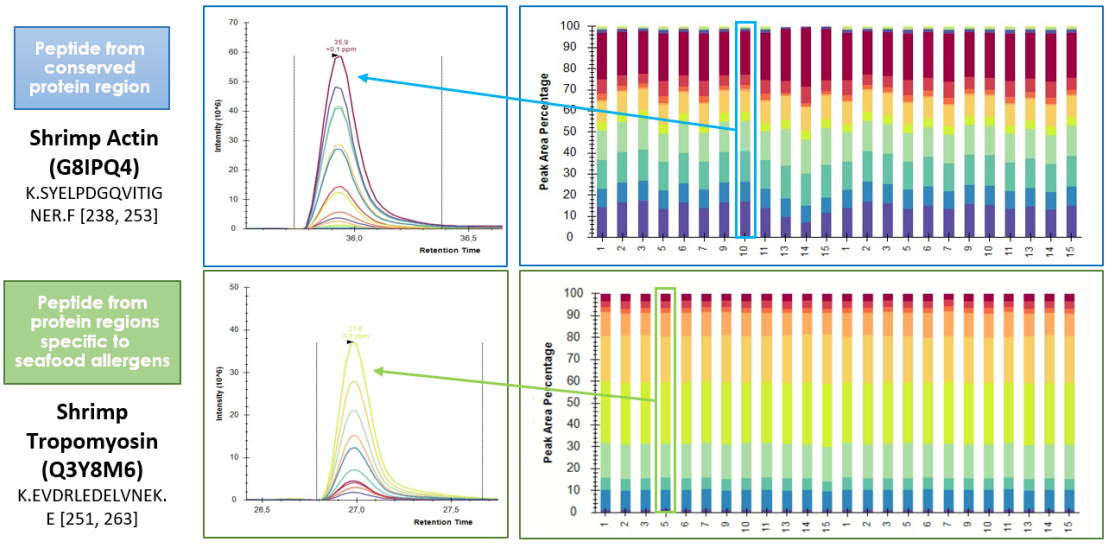

Figure 1. *PRM analysis of peptides extracted through PreOmics columns from 15 different shrimp samples run in duplicates. Peptide peak identification from shrimp Actin (G8IPQ4) conserved region K.SYELPDGQVITIGNER.F [238, 253] (chromatogram from sample 10.1; RT: 35.9+0.1 ppm) and Peptide peak identification from shrimp Tropomyosin (Q3Y8M6) specific region K.EVDRLEDELVNEK.E [251, 263] (chromatogram from sample 5.1; RT: 27.0-0.6 ppm).*