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Development and validation of a sandwich enzyme-linked immunosorbent assay (ELISA) for the quantification of Ara h6 in peanut flour, peanut extract, and patches for epicutaneous immunotherapy (EPIT)

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Background: EPIT to treat peanut allergy is currently in late stage clinical investigation. Validated analytical assays are needed to accurately quantify major allergen in pharmaceutical allergenic products.

Method: Polyclonal antibodies were produced by immunization of rabbits with purified Ara h6. Antibodies were purified by affinity chromatography. A first portion was used as coating antibody and a second part was labeled with biotin for use as detection antibody. The ELISA was optimized for signal-tonoise ratio and assay sensitivity. Matrix specificity was assessed by spiking purified Ara h6 into the different allergen materials: peanut flour, peanut extract, and patch. Validation was designed according to the International Conference on Harmonization Q2 (R1) and using the tolerance intervals approach, for each allergen product. For API and Patch, accuracy part of the validation was applied on reconstituted form with 108 individual determinations (6 concentration levels x 6 series (various operators and days) x 3 determinations per series).

Results: The calibration curve ranges from 0.391 to 12.5 ng/mL Ara h6. No relevant cross-reactivity was observed; neither with the Ara h6-homologous allergen Ara h2 (not detectable; < 0.1%) nor with Ara h1 allergen (0.62%). Using this ELISA, the Ara h6 content in peanut extract was determined to be around 3% (w/w), in line with literature. For Peanut extract quantification, the method is linear (r=0.955), precise (coefficient of variation for repeatability (CVr) < coefficient of variation for intermediate precision (CVR) ≤15%), true (relative bias ≤8%) and accurate over the range 40% to 200% (1.0 to 5.1 % (w Ara h6 / w extract)). For Peanut patch quantification, the method is linear (r=0.998), precise (reconstituted samples: CVr < CVR ≤17%, authentic patch samples: CVr < CVR ≤6%), true (relative bias ≤8%) and accurate over the range 40% to 200% (2.7 to 13.5 µg Ara h6 per 250µg peanut protein patch). Absence of matrix effect was demonstrated for all three tested matrices.

Conclusion: An Ara h6 ELISA was developed for major allergen quantification in peanut products from the source material to the treatment patches. The validation demonstrated that this ELISA is suitable as quantitative assay for release and stability testing.