

## Development of a micro UHPLC-HRMS/MS method for the absolute quantification of adjuvants in wine

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Proteinaceous products are frequently employed as adjuvants during winemaking to improve the wine's clarity, color, flavor, and structural stability. For allergic subjects, the presence of this fining agent traces (such as egg albumen and animal gelatine) in the finished product poses a risk. For this reason, several Directives of European Union require the labeling of allergenic ingredients whenever if they are only used for food making, regardless of their level of inclusion. The International Organization of Vine and Wine (OIV) had first established the limit of detection (LOD) and quantification (LOQ) for milk and egg clarifiers for immunoassay at 0.25 mg/L and 0.50 mg/L, respectively (regulation 579/2012). Quantification of allergen traces in food can be achieved using different techniques, including PCR and ELISA, but both methods do not achieve the results that are promised by reference methods. EU legislation with the Council Directive 96/23/EC's implementation indicated MS techniques as best candidates for this purpose.

Our research's objective is to provide an accurate reference approach for the absolute quantification of adjuvants in red wine using MS techniques. In order to assess the selected peptides for fining agent detection, a HPLC-HRMS DDA (Data Dependent Analysis) on albumen and gelatine standards was carried out, and 5 distinct and specific peptides (3 from albumen and 2 from gelatin) were chosen. The peptides were selected for the absence of both missed tryptic cleavage sites and post-translational modifications. Moreover, they have to be species-specific for each fining agents, as determined by BLAST.

In the current investigation, a set of five signature peptides were preliminary quantified by LC-HRMS/MS up to 0,25 ppm. The workflow will proceed with a) the synthetization of the labeled and unlabeled signature peptides; b) the creation of a calibration curve using synthesized peptides (internal standard) in the range of 0,1 to 10 ppm; c) the validation of this procedure. The synthetic peptides will be employed as calibrants, and the Amino Acid Analysis (AAA) will assign an uncertainty value for each peptide to achieve metrological traceability to the SI.

**Keywords:** traceability, adjuvants, absolute quantification, signature peptides, UHPLC-HRMS/MS.

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