

Chromatography

September 21-23, 2016 Amsterdam, Netherlands

Size-exclusion HPLC as a sensitive and calibrationless method for complex peptide mixtures quantification

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Nowadays, protein hydrolysates and fractions are of great interest because of their nutritional or bioactive properties. The quantification of total peptide concentration is of a crucial importance in order to establish mass balance of fractionation processes. This is commonly done either by Kjeldhal analysis, or by colorimetric assays, whose are laborious and time-consuming. This work describes an original methodology to quantify complex peptide mixtures by size-exclusion high-performance liquid chromatography (SE-HPLC), already used to characterize the molecular weight distribution of hydrolysate. In the proposed methodology, each point of the complex mixture chromatogram is regarded as a mixture of peptides sharing same molar extinction coefficient and molar weight, estimated from its retention time and the hydrolysate aminogram. This allows a conversion of absorbance into concentration (using Beer-Lambert law) and the integration of the overall signal gives the peptide concentration of the analysed fraction. The methodology was first tested on simulated elutions of peptide mixtures and a good estimation of the total peptide concentration was observed (error less than 10%). Then 30 fractions obtained by ultrafiltration of hydrolysates from two different sources were titrated by Kjeldahl or BCA analysis and analysed by SE-HPLC for an experimental validation of the methodology. Very good matches between methods were obtained (error less than 15%). Moreover, the presence of organic solvents or salts in samples does not impact the accuracy of the methodology contrary to common quantification methods.

Biography

Romain Kapel is a third year PhD student from University of Lorraine, France. In 2013, she graduated as a chemical and process engineer. She is now working on the fractionation of protein hydrolysate during her PhD program. She will present an original methodology to quantify peptide concentration in complex hydrolysates by size-exclusion HPLC.

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