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Size exclusion flow-based low-pressure chromatographic approach for the molecular weight profiling in protein hydrolysates

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European Union regulations on feed additives impose strict requirements to ensure safety and quality in animal nutrition, thereby driving the demand for reliable and rapid analytical tools to characterize bioactive compounds in feed matrices [1]. Protein evaluation is predominantly performed using high-performance liquid chromatography (HPLC). Although HPLC is a widely used and robust technique, it is often associated with high operational costs, lengthy analysis times, the need for complex instrumentation and the need specialized operators. To overcome these limitations, this work presents the development of a chromatographic sequential injection analysis (SIA) system that incorporates a laboratory-made size exclusion column. This column was specifically designed to screen compounds across a range of molecular weights, with a particular focus on the discrimination based on the regulated 10 kDa threshold. Integrating this column into the SIA manifold enables a rapid, selective separation and quantification of low-molecular-weight compounds, significantly enhancing analytical throughput and overall process efficiency. This sequential injection chromatography approach offers distinct advantages, including improved precision, full automation, and reduced reagent consumption [2]. Consequently, this method provides a cost-effective, miniaturized, and automated solution with strong potential for real-time control in industrial production environments. This is particularly relevant in the animal feed manufacturing sector, where the accurate monitoring of peptides, amino acids, and other bioactive molecules under 10 kDa is essential to ensure nutritional quality, regulatory compliance, and optimized formulation strategies.

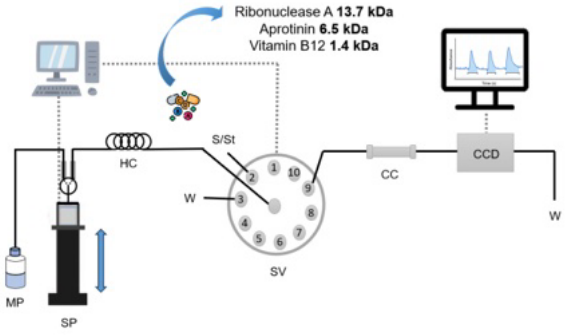

Figure 1: Sequential injection analysis manifold for the molecular weight screening in hydrolysates; MP, Mobile phase; SP, Syringe pump 5 mL; HC, Holding coil 3 m; S/St, Sample/Standard; SV, Selection valve; CC, Chromatographic column; CCD, UV-Vis detector; W, waste.

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