Comprehensive Validation of High-Performance Liquid Chromatography-Diode Array Detection (HPLC-DAD) for Quantitative Assessment of Caffeic Acid in Phenolic Extracts from Olive Mill Wastewater

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Abstract : In this study, it introduce and validate a high-performance liquid chromatography method with diode-array detection (HPLC-DAD) specifically designed for the accurate quantification of caffeic acid in phenolic extracts obtained from olive mill wastewater. The separation process of caffeic acid was effectively achieved through the use of an Acclaim Polar Advantage column (5µm, 250x4.6mm). A meticulous multi-step gradient mobile phase was employed, comprising water acidified with phosphoric acid (pH 2.3) and acetonitrile, to ensure optimal separation. The diode-array detection was adeptly conducted within the UV-VIS spectrum, spanning a range of 200-800 nm, which facilitated precise analytical results. The method underwent comprehensive validation, addressing several essential analytical parameters, including specificity, repeatability, linearity, as well as the limits of detection and quantification, alongside measurement uncertainty. The generated linear standard curves displayed high correlation coefficients, underscoring the method's efficacy and consistency. This validated approach is not only robust but also demonstrates exceptional reliability for the focused analysis of caffeic acid within the intricate matrices of wastewater, thus offering significant potential for applications in environmental and analytical chemistry.

Keywords : high-performance liquid chromatography (HPLC-DAD), caffeic acid analysis, olive mill wastewater phenolics, analytical method validation

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