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PROTEIN SEPARATIONS USING ENHANCED-FLUIDITY LIQUID CHROMATOGRAPHY

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Highlights:

- Gradient enhanced-fluidity liquid chromatography (EFLC) of proteins was illustrated.
- First use of EFLC for separation of hydrophilic, large proteins
- Thirteen protein analytes were separated in under 5 minutes.
- EFLC offered different selectivity for a protein pair relative to the HPLC method.

Abstract:

Enhanced-fluidity liquid chromatography (EFLC) methods using methanol/H₂O/CO₂ and hydrophilic interaction liquid chromatography (HILIC) were explored for the separation of proteins and peptides. EFLC is a separation mode that uses a mobile phase made of conventional solvents combined with liquid carbon dioxide (CO₂) in subcritical conditions. The addition of liquid CO₂ enhances diffusivity and decreases viscosity while maintaining mixture polarity, which typically results in reduced time of analysis. TFA additive and elevated temperature were leveraged as key factors in the separation of a 13-analyte intact protein mixture in under 5 minutes. Under these conditions EFLC showed modest improvement in terms of peak asymmetry and analysis time over the competing ACN/H₂O separation. Protein analytes detected by electrospray ionization – quadrupole time of flight, were shown to be unaffected by the addition of CO₂ in the mobile phase. Herein, the feasibility of separating hydrophilic proteins up to 80 kDa (with transferrin) is demonstrated for CO₂-containing mobile phases. **Keywords:** Hydrophilic interaction liquid chromatography; HILIC; enhanced-fluidity liquid chromatography; proteins; peptides; subcritical chromatography

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