Title: PROTEIN SEPARATIONS USING ENHANCED-FLUIDITY LIQUID CHROMATOGRAPHY

Authors: Raffeal Bennett, Susan V. Olesik

PII: S0021-9673(17)31061-0
DOI: http://dx.doi.org/doi:10.1016/j.chroma.2017.07.060
Reference: CHROMA 358707

To appear in: *Journal of Chromatography A*

Received date: 2-5-2017
Revised date: 17-7-2017
Accepted date: 18-7-2017

Please cite this article as: Raffeal Bennett, Susan V.Olesik, PROTEIN SEPARATIONS USING ENHANCED-FLUIDITY LIQUID CHROMATOGRAPHY, Journal of Chromatography Ahttp://dx.doi.org/10.1016/j.chroma.2017.07.060

PROTEIN SEPARATIONS USING ENHANCED-FLUIDITY LIQUID CHROMATOGRAPHY

Raffeal Bennett and Susan V. Olesik*
Department of Chemistry and Biochemistry, The Ohio State University,
100 West 18th Avenue, Columbus, OH 43210, United States of America
*Corresponding author: Phone: (614) 292-0733, Fax: (614) 292-1685
e-mail: olesik.1@osu.edu

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/H_2O/CO_2 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 (CO_2) in subcritical conditions. The addition of liquid CO_2 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/H_2O separation. Protein analytes detected by electrospray ionization – quadrupole time of flight, were shown to be unaffected by the addition of CO_2 in the mobile phase. Herein, the feasibility of separating hydrophilic proteins up to 80 kDa (with transferrin) is demonstrated for CO_2-containing mobile phases. **Keywords:** Hydrophilic interaction liquid chromatography; HILIC; enhanced-fluidity liquid chromatography; proteins; peptides; subcritical chromatography