TECHNICAL BRIEF

Supplementary Material

Improved protocol for chromatofocusing on the ProteomeLab PF2D

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Method

Human serum (100 µl) was centrifuged at 4 °C for 20 min at 16,000 x g and the protein concentrations determined in the supernatant using the BioRad protein assay reagent. Samples were diluted with 'start' buffer (6 M urea, 25 mM BisTris, 0.2% OG, pH 8.5) to obtain the desired protein concentration and filtered through 0.22 µm Millipore Express PES Membrane filters. Sample analysis with the ProteomeLab PF2D™ (Beckman-Coulter, Fullerton, CA, USA) was performed by first equilibrating the chromatofocusing column (first dimension) with start buffer for 210 min. The pH of the effluent was monitored and equilibration was considered complete when the effluent had a stable pH of 8.5 ± 0.1. Following loading of 2 ml (1.5 mg) of sample on the chromatofocusing column (HPCF-1D column, 250 x 2.1 mm, Eprogen, Darien, IL, USA), the baseline was again allowed to stabilize for 20 min before elution with a pH gradient. The pH gradients (internal and isocratic) were formed using 'start' buffer and 'elution' buffer (6 M urea, 10 % (v/v) Polybuffer™ 74 from GE Healthcare, 0.2% octylglucoside, pH 4), prepared essentially as described [1]. Buffers were filtered through 0.2 µm nylon membrane filters (NALGENE) before use. Flow rate was 0.2 ml/min and column temperature 20 °C. Chromatofocusing profiles were monitored at 280 nm. Fractions were collected approximately every 0.3 pH units using a model SC 100 fraction collector. Elution with a pH gradient was followed by washing of the chromatofocusing column with 1 M NaCl, 0.2 % octylglucoside, for 40 min. Second dimension separations were carried out by reverse-phase chromatography on a ODS-IIIE C18 column (33 x 4.6 mm, 1.5 µm nonporous silica beads, Eprogen). From fractions of the first dimension, 200 µl were injected into the second dimension column and eluted at a flow rate of 0.75 ml/min with 0 to 100% linear gradients of solvent A (0.1 % (v/v) trifluoroacetic acid) and solvent B (0.08 % (v/v) trifluoroacetic acid in acetonitrile) for 35 min. The column temperature was maintained at 50 °C and the absorption of the effluent was monitored at 214 nm.

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Reference

[1] Zheng, S., Schneider, K.A., Barder, T.J., Lubman, D.M., *Biotechniques* 2003, *35*, 1202-1212.

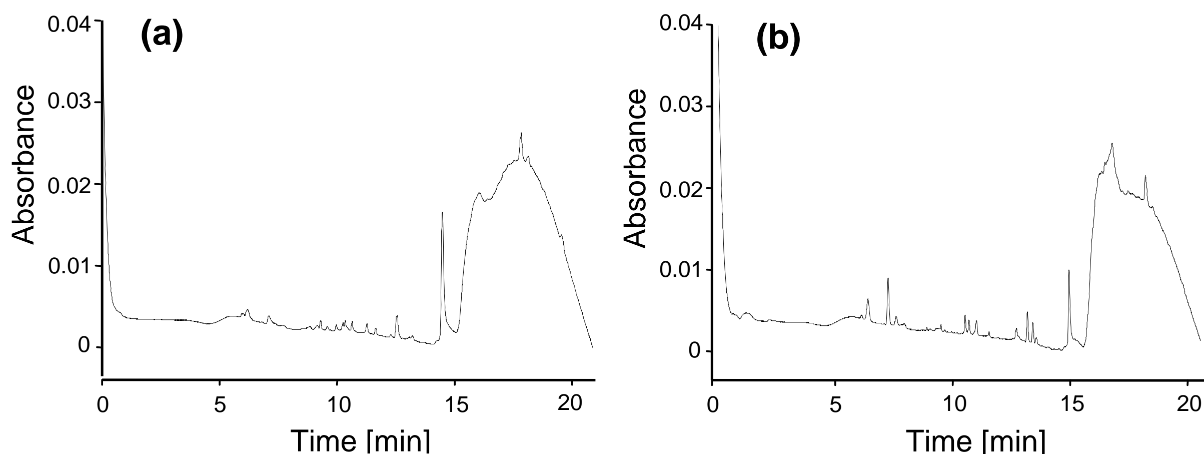

Figure 1. Elution profiles of reverse phase chromatography (second dimension) of the chromatofocusing flow-through (7.5 to 15 min) of run one (a) and run 18 (b), performed on the same chromatofocusing column. The elution profiles were monitored at 214 nm. See Methods for other details.

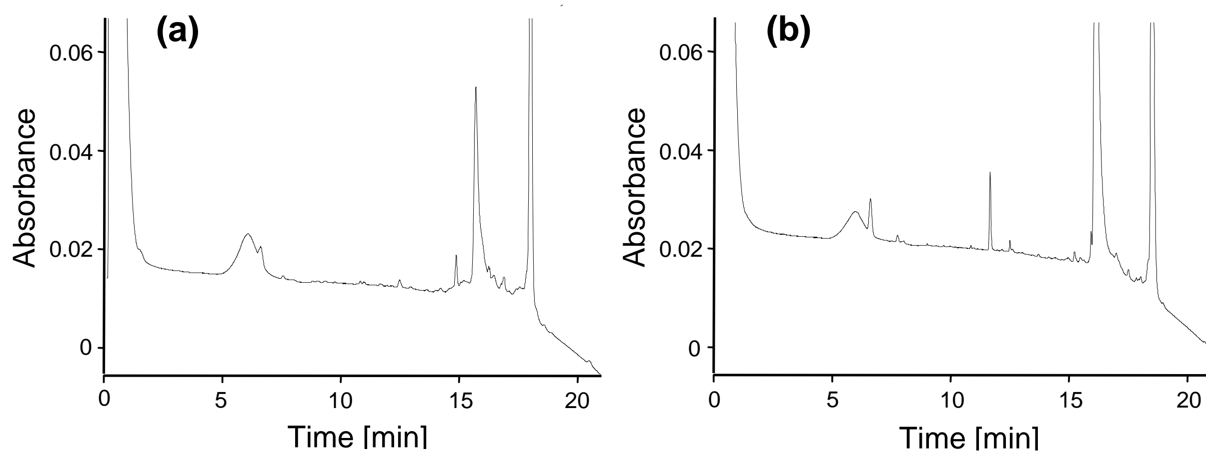

Figure 2. Elution profiles of reverse phase chromatography (second dimension) of the chromatofocusing fractions covering the pH range 5.56-5.26 (102.4-109.5 min) of run one (a) and run 18 (b), performed on the same chromatofocusing column. The elution profiles were monitored at 214 nm. See Methods for other details.

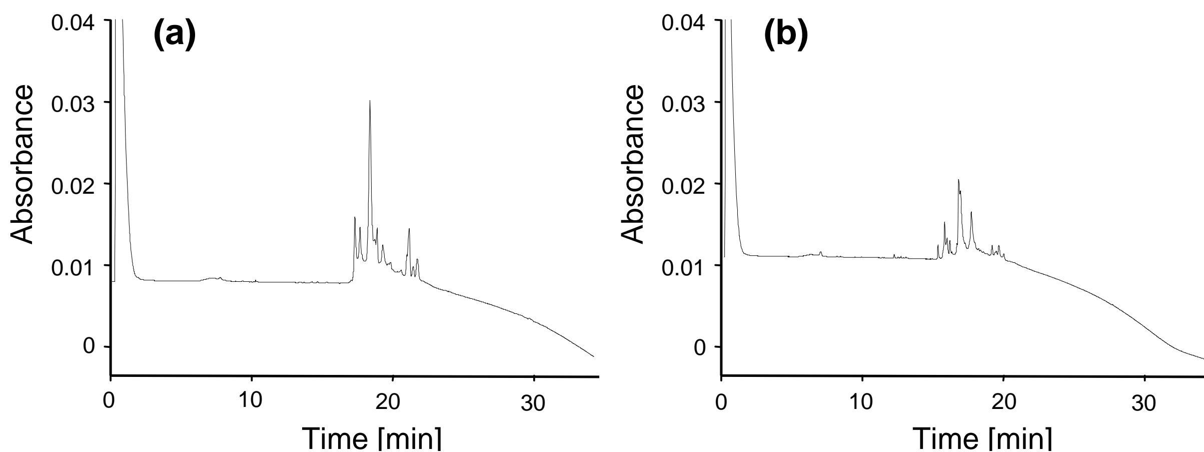

Figure 3. Elution profiles of reverse phase chromatography (second dimension) of the chromatofocusing column salt wash (123-130 min) after run one (a) and run 18 (b), performed on the same chromatofocusing column. The elution profiles were monitored at 214 nm. See Methods for other details.