

application note

high-speed determination and examination of high molecular weight proteins with the expressLC™ system

reversed-phase analysis of antibodies and other large proteins in minutes, not hours!

introduction

While the liquid chromatographic separation of peptides has been successfully accomplished for at least two decades, the RP separation of large biomolecules, such as antibodies, fusion proteins, and enzymes has lagged in development. Peptides look structurally and act chromatographically much like small drug molecules; that theoretical and empirical base has extensive literature for the analyst to employ for a given analytical situation. With the advent of biotechnology and the dearth of analytical techniques to examine and characterize protein-based therapeutic agents, RP of large proteins has been studied more intensely. In the usual case, the standard RP method, using a gradient consisting of water, acetonitrile and trifluoroacetic acid is capable, at a minimum, of determining the protein concentration and a retention time characteristic, but not unique, to the species of interest. In the most favorable cases, impurities can also be monitored and the populations of the various glycoforms can be observed. For these reasons, the RP gradient determination of protein molecules has become a standard assay as part of most biologic drug species characterizations.

figure 1. extended-run analysis of a proprietary monoclonal IgG

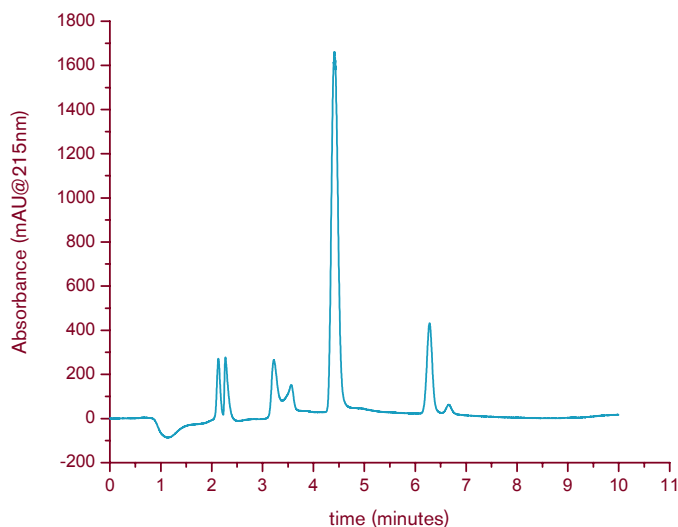
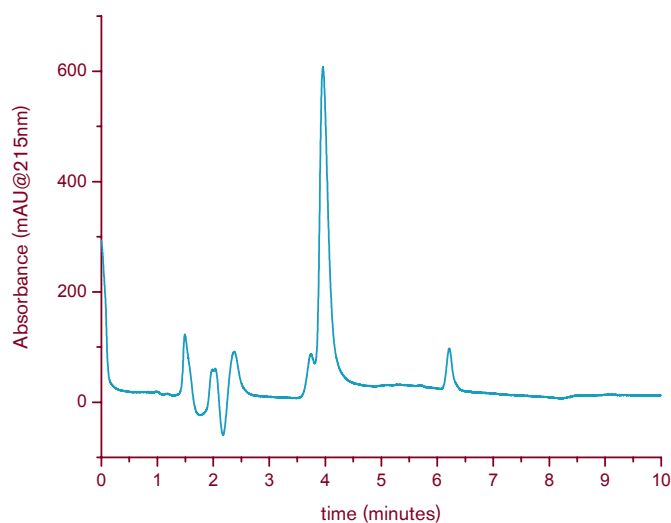


figure 2. extended-run analysis of a polyclonal rabbit IgG



faster, higher resolution protein analysis with the expressLC system

The standard RP separation of large protein molecules has suffered from characteristically long analysis times. While not universally accepted, the prevalent theoretical model for mobile phase/stationary phase interaction is consistent with the observed experimental conditions required for optimal separation. In the current model, the protein is kept in solution by the hydrophilic groups on the exterior of the protein's native conformation. As the protein is exposed to increasingly higher acetonitrile concentrations, the tertiary structure is disturbed and the protein starts to unfold, exposing the inner hydrophobic amino acid sequences. It is the inner, hydrophobic structure which pushes the aqueous/stationary phase equilibrium towards lower mobile phase solubility and allows interaction with the hydrophobic (C18, C8, C4) stationary phase. It is believed that the protein molecule is then released by an entropy-driven, endothermic process at a higher acetonitrile concentration. It is important to understand this mechanism in which the mobile phase and several other external variables are responsible for *the attachment, residence time, and release of large protein molecules* in RP chromatography, is unlike the chromatography of small drug molecules. The conditions required for analysis require *very slow flow rates*, in part to allow analyte/stationary phase interaction, and the chromatographic peak profiles are nonetheless often lacking in efficiency and selectivity. The precise gradient control of the ExpressLC system allows a drastic reduction in analysis time as compared with the conventional LC times of 15-90 minutes, not including re-equilibration time. The superior gradient control and the fast (1-5 minute) re-equilibration times of the ExpressLC system allow analysis of large molecules in minutes.

figure 3. high-speed goat IgG concentration determination

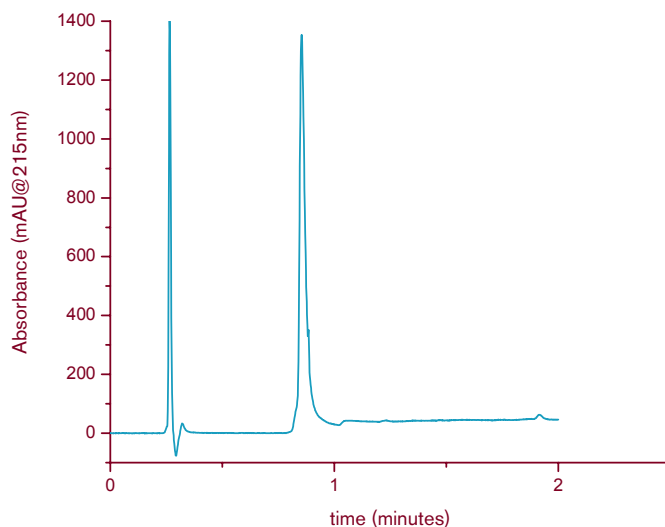
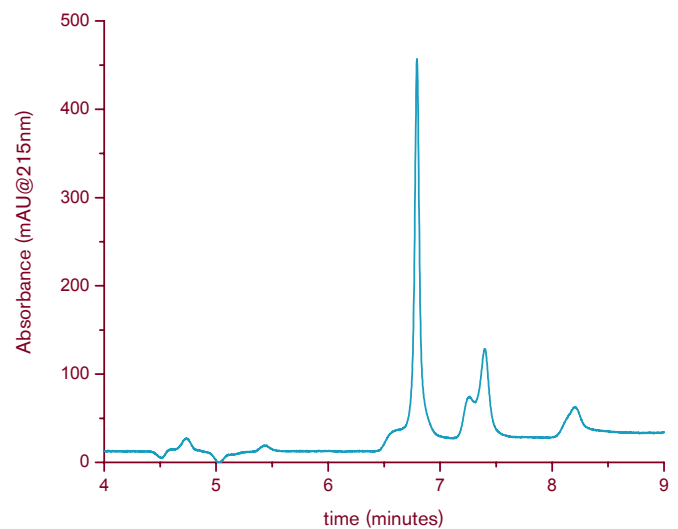


figure 4. extended-run proprietary 92 kD enzyme purity analysis



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expressLC system specifications**configuration**

expressLC-100 Single-channel System: Includes binary gradient pump, electronic injection valve, column temperature control, and array-based UV detection system. Optional high-speed autosampler available.

expressLC-800 8-channel Parallel System: Includes 8 binary gradient pumps, 8 electronic injection valves, 8 column temperature control compartments, an array-based UV detection system and high-throughput autosampler.

flow rate range

0.20–30 $\mu\text{L}/\text{min}$

pump type

Microfluidic direct pumping system with independent flow control feedback for each mobile phase. Retention time RSD < 0.5%.

gradient formation

High pressure gradient mixing. System can run full gradients as rapidly as 8 seconds. Maximum gradient length 2 hrs. at 5 $\mu\text{L}/\text{min}$.

delay volume

< 500 nL from mixer to column.

mobile phase compatibility

All mobile phases compatible with 316 stainless steel, PEEK, and silica.

injection valve

Eksigent Variable-Volume Injection System (software selectable). Standard injection volume 10–250 nL (larger injection volumes available).

columns

System optimized for 2.5–15 cm, 300 μm i.d. capillary LC columns

column temperature control

Software selectable from 27–40°C; stability within $\pm 0.1^\circ\text{C}$

detection

UV absorbance detection from 200–380 nm using linear CCD array detector. Detector drift $\leq 4 \times 10^{-4}$ AU/hr Non-linearity $\leq 5\%$ @ 2 AU.

flow cell

45 nL microfabricated flow cell with integral fiber optics, 4 mm path length

autosampler

High-throughput CTC autosampler available

system control

Computer with graphical user interface for control of all system parameters. Software allows import of run tables and creates CDF, text, and Excel files for data export and analysis. Tracking of instrument runtime, column usage, total injections, solvent usage, lamp hours, and error codes. System drivers available for Thermo Electron's Xcalibur and Applied Biosystems/MDS SCIEX Analyst 1.4.1 mass spectrometer software.

report features

Generates reports that include method conditions, chromatograms, peak retention times and areas, and spectral absorbance map.

dimensions**expressLC-100 System:**

21" (53 cm) wide, 20" (51 cm) deep, 18" (46 cm) high

expressLC-100 Autosampler:

Additional 14" (36 cm) high and 6" (15 cm) wide

expressLC-800 System:

30" (76 cm) wide, 34" (86 cm) deep, 40" (102 cm) high

expressLC-800 Autosampler:

Additional 16" (41 cm) high and 16" (41 cm) wide

computer

Additional lab space needed for keyboard, mouse and monitor