

application note

use of the eksigent expressLC™ system for peptide separations

rapid, high resolution separations accelerate peptide mapping analysis

introduction

Peptide maps are particularly useful for comparison of different lots of a therapeutic protein and for use as the first dimension for inference of the protein's amino acid sequential structure, degrees and sites of glycosylation, and extent of post-translational modification. After hydrolysis of the protein, the resulting peptides (ranging in size from a few to several hundred amino acid residues) are separated using HPLC and the resulting chromatogram (peptide map) is analyzed. Crucial to the protocol is the ability to separate the peptides with high retention time precision so that comparison of maps does not regularly require an identification step, such as mass spectrometry, cross-reaction with antibodies, or partial sequencing. As demonstrated in the chromatogram below, the ExpressLC system, with its characteristic high-accuracy pumping profile, is ideal for fast and repeatable analysis of peptide standards and samples.

results

The chromatogram below illustrates the sharp peaks that the ExpressLC system generates, even for rapid gradient separation of this complex sample. The precision of the Microfluidic Flow Control (MFC) system allow excellent retention time and peak area precision, which are both critical attributes to effective peptide map analysis.

figure 1. chromatogram of peptide separation

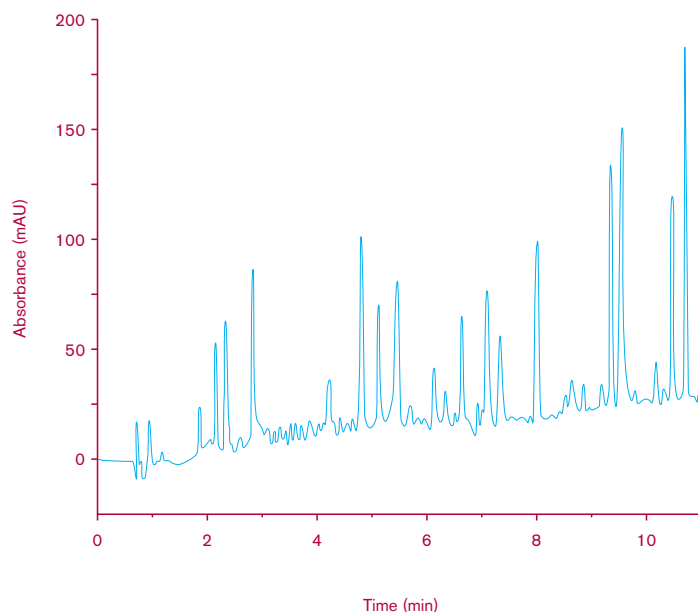


figure 2. experimental conditions

Instrument:	ExpressLC-100 system with CTC LC PAL autosampler
Column:	Discovery C18, 0.32 mm, 10 cm long
Mobile Phase:	A/B water/acetonitrile
Flow rate:	7 μ L/min
Gradient:	1 to 53 % acetonitrile 20 min, 53 to 98% acetonitrile 25 min
Injection:	150 nL injection of Tryptic Anti-Factor IX



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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