UFFLC of the Proteome

Ultra High Pressure and Ultra Fast Flow Liquid Chromatography: A New Approach To Proteomics

Technology to Change the Proteome Paradigm

The Ultra High Pressure (UHP) Liquid Chromatography System provides reproducible high-speed separations of intact proteins in minutes. This technology, when coupled with existing methods, allows a new approach to Proteomics. Imagine high resolutions native protein separations in under 10 minutes, with resolving power that would normally require one hour. In addition, the system facilitates loading that allows multiple paths of investigation on the collected protein fractions, and the ability to run multidimensional separations with orthogonal feed back to mine information never before available.

Orthogonal Confirmation of Proteins

The use of a top down approach and the traditional MudPIT approach allows confirmation of a hit through MudPIT identification with a native protein MW. If the protein is not found in the fraction, the possibility of post translational changes or other modifications can be explored with secondary separations and MS/MS techniques to identify the proteins in the suspect fraction.

Increased Sensitivity of Low Abundance Proteins

The use of the top down fractionation of proteins will dramatically increase the sensitivity of low abundance proteins as shown in the following figure. The abundant proteins will no longer mask the low abundance proteins. The column loading can be calculated to allow extremely low abundance proteins to be seen in the Mud PIT or MS/MS scans of the native protein.

Resolution of Post Translational Changes

The use of a secondary separation on a fraction will allow for further definition of what is occurring in the proteome by allowing post translational modifications to be identified by either MS/MS or further chromatography separation.

Faster Proteome Library Generation

Native protein separations in minutes with the ability to collect fractions and use multidimensional and orthogonal means of protein identification will allow libraries to be developed in a faster manner than a single approach. Leave the MudPIT and see the light of Top-down and Bottom-up Proteomics.
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See the Speed and Resolution Afforded by UFFLC

The yeast Lysate separation below on a 5 µm Cation Exchange Column shows a reduction in separation time from 45 minutes (with a 20 min regeneration time) to 8 minutes (2 min regeneration). The second chromatogram shows the increased resolution of the faster run.

UFFLC Effect on Kinetics in Separations

The Proof is in the Chromatography

As the data presented shows the separations are much faster than conventional chromatography but what if time is extended does resolution increase? The data below shows the effect of increased analysis time. Note there are more peaks in the UFFLC run.

How it Works

The following graphic shows the effects of high pressure and flows that are 5-6 times faster than a standard chromatographic process. This allows the use of 3 and 5 µm particles, with separations expected with smaller particles. The same is true if sub 2 µm particles are used at high flow. The system must have the flow and pressure specifications to meet these demanding requirements.

Only LabAlliance (a division of Scientific Systems, Inc.) has a system for UFFLC

The combination of our Ultra High Pressure Pump (up to 18,000 p.s.i.) and flows up to 5ml/min makes UFFLC possible. The UHP autosampler, 20Hz detection and EZStart data and control make the high speed work possible. Other UHP systems cannot provide the required pressures and flows on 10 to 15 cm small particle columns to perform UFFLC.
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UHP PUMP SPECIFICATIONS
Flow Rates 0.001 - 5.000 mL/min. (each pump)
Pressure 0 – 18,000 psi
Pulsation ± 1% @ 1mL/min. and 1,000 psi
Pressure Accuracy ± 1% of full scale pressure
Flow Accuracy ± 2%
Flow Precision 0.2% RSD
Gradient Accuracy Within 1% absolute @ 1mL/min. and 10,000 psi
Voltage 110-120 Vac, 50/60 Hz; or 220-240 Vac, 50-60 Hz
Physical 6.5” H x 12.0” W x 15.5” D (165 x 787 x 394 mm); 42 lbs (19 kg)

GRADIENT CONTROL
- Gradient control is achieved via host PC with EZ Start software.
- Communication from Pump Module to PC is through an RS-232 card.
- Typical Pump Gradient Percent Range:
  - 0-100% in 0.1% increments.
  - Low volume mixer is included

Specifications subject to change without notice.

UHP Reproducibility Study – 4 Hemoglobin FACS runs

MODEL 525 DETECTOR SPECIFICATIONS
Optical design Concave holographic grating monochromator, with dual beam optics; pre-aligned lamps
Noise <±1.0 x 10⁻⁵ AU @254 nm, 1.0 s rise time; <±2.5 x 10⁻⁵ AU @254 and 280 nm, 1.0 s rise time, dual-wavelength mode (525 only)
Drift <2 x 10⁻⁴ AU/hr. after warm-up @ 254 nm
Wavelength range
  - Deuterium (D2) lamp: 190-365 nm
  - Tungsten (W) lamp: 366-800 nm
  - Dual Wavelength: 190-450 nm, or, 366-800 nm
Lamp(s) D2 and W standard (525)
Wavelength accuracy ±1.0 nm
Wavelength precision ±0.1 nm
Spectral bandwidth 6 nm
Absorbance range 0.0005-3.0 AUFS
Absorbance linearity>1% to 2.0 AU @254 nm
Remote inputs Run, Stop, Zero
Output flags Ready, Accessory Relay
Analog outputs (2): both range-selectable over entire absorbance range (525); 1.0 AU/V and range-selectable (201)
Ambient Environment 10 to 40° C; 5% to 95% relative humidity, noncondensing
Dimensions 17 cm x 30 cm x 41 cm (HxWxL)
Weight 14 kg (31 lbs.)

UHP Autosampler
Injection range
- 1 ul - 5000 ul; 1 ul increments;
- 500 ul syringe
- UHP model has 10 ul loop

Sample capacity
- 2 well plates 96 or 384 (deep or shallow);
- 96 vials (1.5 ml); Option: 24 vials (10 ml)
Injection precision
RSD for injection volume > 10 ul
Full-loop: 0.3%; Partial-loop: 0.5%;
ul-pick-up: 1.0%

Carry-over
as flush-out characteristic for a nonsticking compound
< 0.05% (Uracil in water; with 300 ul wash)

Needle wash
1 solvent; programmable volume;
wash reservoir: 250 ml

**EZ Start SPECIFICATIONS**

What is EZStart?

EZStart is a complete data acquisition, processing, and control package for 1 instrument. Based on the highly popular EZChrom *Elite*™ software package, EZStart is the ideal solution for laboratories who want a simple-to-use chromatography data system with comprehensive software features and instrument control. It is intended to provide the basic requirements for data acquisition and optional instrument control for a single workstation. EZStart consists of two software modules: **EZStart Configuration** and **EZStart**. With EZStart, instrument control and analysis is reduced to just 3 steps: **Configure, Run, and Report**.

Once the instrument is configured, any user can launch EZStart, immediately choose a method and submit samples for acquisition. All this operation is done in EZStart’s Single Window Access environment - a design that greatly simplifies tasks for the casual or novice user. Either single runs or sequence runs can be performed. Users can still take advantage of the intelligent features in SmartSequence™, a powerful tool that automates much of the decision making process based on user defined criteria. If methods or sequences need to be created from scratch, the simple wizards will guide you through a stepwise process.

Flexible report management

Within any method exists a report template. Users simply select from standard templates and can produce the reports they require. EZStart’s custom report editor may be used to modify existing templates or to create new report templates.

What can EZStart do for you?

Here are just a few of the benefits:

- Takes you from configuration to analysis in just 3 steps.
- Facilitate productivity further with Method and Sequence wizards.
- Respond automatically and intelligently to real-world conditions with SmartSequence™ technology.
ORDERING INFORMATION

Basic System: Binary UHP gradient chromatography system, *includes*:
- 2 UHP pumps (SS);
- EZ Start software and PC;
- Model 525 programmable variable dual channel UV/Vis detector;
- System accessories kit
- Autosampler and EZ Start Driver

Contact your representative for a system quote.

**2-Minute Hemoglobin Rapid Screen**

SAMPLE: Hemoglobin FASC standard
COLUMN: PolyCAT A, 100x2.1-mm, 3-µm, 1500-Å  FLOW: 1.2 ml/min  A415  Pressure: 6938 psi
GRADIENT: 0-0.7': 18-55% B; 0.7-1.1': 55-85% B; 1.1-1.2': 85-100% B; 1.2-1.3': 100% B; 1.4': Initial
A) 40 mM Bis-Tris + 2 mM KCN, pH 6.5  B) 40 mM Bis-Tris + 2 mM KCN + 200 mM NaCl, pH 6.8

LabAlliance will shorten your protein separation times