C4 Reverse Phase Columns for the cHiPLC®-Nanoflex System

Robust, Higher Throughput Separations by cHiPLC nanoflow LC MS

The analysis of intact proteins by mass spectrometry is most often done using reverse phase separation in front of an electrospray mass spectrometer. Protein formulation buffers are designed to keep intact proteins stable in solution, however they often contain components which are very unfriendly to MS analysis. Therefore, desalting of the protein sample is critical to obtain high quality MS spectra necessary for intact protein mass deconvolution. In addition, this application is often done in laboratories where there are larger numbers of samples for analysis and having an on-line sample clean-up and analysis method is advantageous for higher throughput.

Proteins are much more hydrophobic than peptides and other smaller molecules due to their size. Better separation is typically achieved by using a phase that has a shorter alkyl chain than typical C18 reverse phase chromatographic media. Availability of C4 media with 300 Å, 5 µm particles packed in a microfluidic chip enables easy switching from peptide or small molecule analysis workflows typically done on C18 phases to intact protein analysis, while staying in the nanoflow regime.

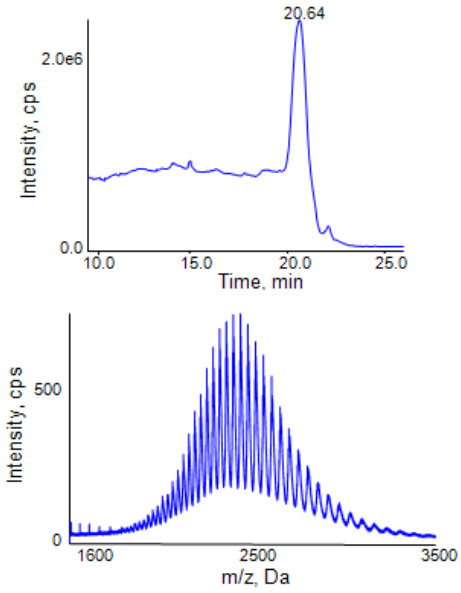

Figure 1. Analysis of Anti-Actin IgG Protein. The intact protein (120 fmol on column) was separated on the C4 nanoflex chip using a 15 min gradient (top). The LC peak at 20.64 mins contained the IgG TOF MS spectrum (bottom).

Advantages of the cHiPLC®-NanoFlex and the cHiPLC Technology

- **Easy:** Plug & play chip simplicity with the sensitivity performance of a nanoflow column
- **Extendable:** Flexibility to switch between workflows and projects rapidly in multi-user labs
- **Every time:** Reproducible results from day-to-day, column-to-column and lab-to-lab

Analysis of Intact Proteins by cHiPLC/MS

The C4 wide pore reverse phase was used on the cHiPLC®-Nanoflex System in front of a TripleTOF™ 5600 System (AB SCIEX). The nanoflex was configured in trap-elute mode to allow for increased desalting of the protein before injection into the MS system. Holding the temperature of the chip between 45-55 ºC provided the best separation and TOF MS spectra. The gradient for the short IgG separation was a 15 min ramp from 8-40% acetonitrile in 0.1% formic acid at 500 nL/min, then the column was washed for a short time in 90% acetonitrile (Figure 1, top).

Table 1. C4 Reverse Phase cHiPLC®-Nanoflex Chip Options.

Description	Dimensions	Stationary Phase	Flow Range	Part Number
C4 cHiPLC column	15 cm x 75 µm	*ChromXP C4-CL 3 µm, 300 Å*	*200 – 500 nL/min*	*804-00018*
C4 cHiPLC trap	0.5 mm x 200 µm	*ChromXP C4-CL 3 µm, 300 Å*	*1 – 5 µL/min*	*804-00019*

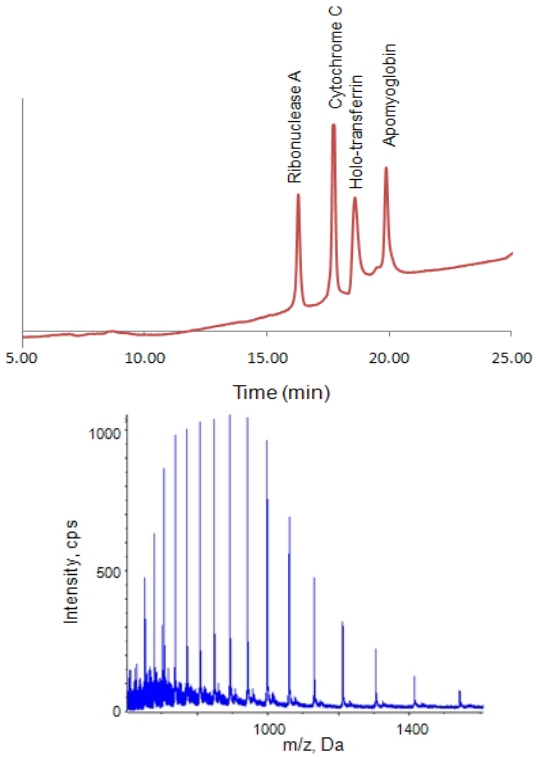

Figure 2. LC Separation of Four Standard Proteins. The UV trace for the separation of four standard proteins is shown (top). The TOF MS spectrum for the last eluting peak gave a reconstructed mass equivalent to the mass of apomyoglobin at m/z 16950 Da (bottom).

A mixture of four standard proteins was analyzed on the C4 chip set (column and trap) to demonstrate the separation power of this chromatographic resin for intact proteins (Figure 2, UV trace). Sufficient separation was obtained to enable extraction of the unblended TOF MS spectra for each protein. The TOF MS spectrum for the protein, apomyoglobin, the late eluting peak at 20 minutes is shown (Figure 2, bottom).

Summary

- The C4 resin, with its shorter alkyl chain to reduce hydrophobicity, is the resin of choice for LC separation of intact proteins
- Higher sensitivity is achieved using the C4 nanoflow chips than the typical high flow applications, key when limited sample is available.
- Availability of this phase in the nanoflex chip format allows the LC MS user to easily switch between peptide identification and intact protein workflows.

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