

## High Throughput Analytical Proteomics Using PicoFrit® Columns

Trypsin in gel digestion of proteins from a single band of a 1D SDS-PAGE can yield a very complex mixture of peptides. Depending on the starting material, these peptides can easily be present at trace levels near the detection limits of a mass spectrometer. This places an importance method sensitivity. The current accepted way to examine complex mixtures of peptides is through reversed phase chromatography with an HPLC linked to a mass spectrometer. A small internal diameter capillary column should be used in order to obtain the best sensitivity from a chromatography standpoint. A novel column called a PicoFrit® column (Figure 1) is currently commercially available with a 75 µm internal diameter. For the best chromatographic resolution a 10 cm packing filled of either BioBasic® or ProteoPep® should be used with a shallow gradient.

The optimal flow rate for a 75 µm PicoFrit column is between 200 and 300 nL/min. To obtain such low flows without a nano-LC, a flow splitter can be made using a zero dead volume Valco tee with several meters of fused-silica tubing on the waste side of the tee.

In order to achieve the maximum lifetime from a PicoFrit column, attention must be paid to the purity of the solvent used for the chromatography. The highest purity acetonitrile and HPLC grade, distilled in glass, water should be used. Filtered water has shown to cause problems. Micro fines of amorphous carbon particles can escape from the activated carbon cartridge and will rapidly clog a column. These particles are not filtered even by 0.2 µm filters. The 18 Mohms resistivity only means that there are a limited amount of ions within the water. Filtered water could contain as much as 100 µg/L of amorphous carbon leaching from the activated carbon cartridge. Mobile phases should be degassed by an ultrasonication for 20 min. Filtration should be avoided due to contaminating substances which can be extracted from membrane filters.

If the HPLC system has been contaminated with filtered water, it might take several weeks after switching to distilled water before getting rid of the contamination. However, until the system is decontaminated, a clogged column can be salvaged by cutting out the carbon plug under a microscope. The column can then be reconnected with a PTFE sleeve.

A clogged column can be diagnosed by observation of the HPLC backing pressure. After installing and conditioning the column, it is important to note the backpressure at the beginning of the gradient as well as the highest acetonitrile concentration and continue to verify that backpressure regularly. If the backpressure starts to rise, this could be due to the clogging of the column. If this is the case, the accumulated carbon can be easily seen through the polyimide coating of the column. Contaminated water can also be diagnosed by examining the sampling cone on the mass spectrometer. With clean water, the sampling cone should remain shiny for weeks. Conversely, if filtered water is

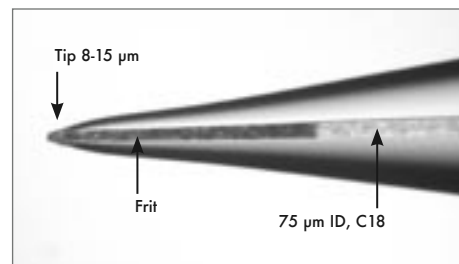


FIGURE 1 The PicoFrit® format incorporates a chromatographic column with a nanospray emitter. This combination tip eliminates band broadening associated with the dead volume normally between the column and the spraying tip.

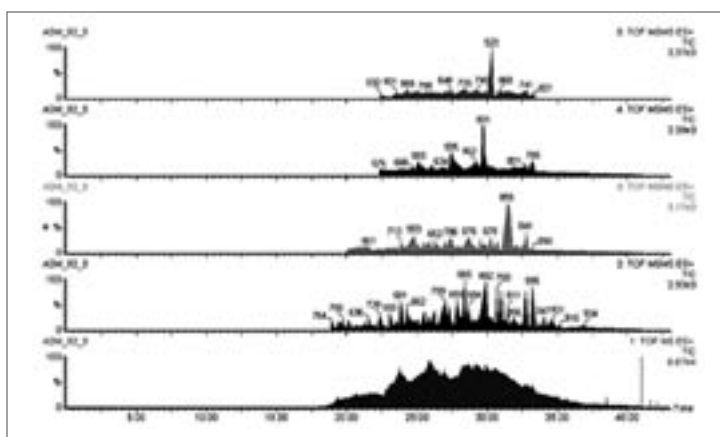


FIGURE 2 In gel digestion of the smooth endoplasmic reticulum on a PicoFrit® column after 928 injections

used, a brown substance will buildup rapidly on the cone surface. The brown material is composed of polyaromatic hydrocarbons (PAH) and other organic molecules desorbed from the carbon particles by the acetonitrile gradient.

After several months of continuous use, the column will stop spraying due to the erosion of the spraying tip. Continuous spraying along with high voltage will dull the sharp edges of the tip and impair the efficiency of the electrical field. The optimal spraying voltage will increase to a high potential and the column should be changed. Typically, in our lab it would take at least 2 months. If handled properly, a 75 µm PicoFrit column can last for several hundred injections of in gel digest protein samples. The column presented here lasted for 1300 injections without losing good peak shape (Figure 2).

Provided by Daniel Boismenu  
Montréal Network for Pharmacoproteomics and Structural Genomics  
McGill University  
Montreal, Quebec, Canada

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