Sample Trap Injection for Improved MS Spectra & Hardware Longevity

Optimizing sensitivity, spectral quality, and hardware longevity for nanobore ESI-MS applications depends largely on sample composition and purity. Where many factors require consideration in obtaining a desirable spray (see Technical Note PF-4, "Spray Optimization"), signal-suppressing salt removal and pre-injection analyte concentration can further enhance spectroscopic data collection and even extend the service time of exisiting hardware.

Advantages of sample trap injections

New Objective provides 2.5 cm sample trap columns available in 75, 100, and 150 µm inner diameters (IDs). Columns are packed with New Objective's premier chemistries: ProteoPep II™, ProteoPep™, BioBasic®, AQUASIL, or strong cation exchange (SCX). With the exception of AQUASIL, all sorbents have an average particle size of 5 µm and a 300Å pore size. AQUASIL has a pore size of 100Å. Custom column packing is available by special order. The column is inserted into the UpChurch® Scientific Trap Cartridge prior to its installation in an online configuration. See Technical Note IF-2 on using the cartridge with the sample trap.

Because nanobore ESI-MS applications measure analytes at subfemtomole levels, obtaining requisite sample concentration for analyte detection is of paramount concern. Sample injection onto a sample trap promotes analyte adsorption onto the reverse-phase sorbent, thereby concentrating it within the trap. In addition, ionic species which can bind to the analyte and suppress its detection are washed away from the adsorbed sample and jettisoned via the waste outlet. While optimal wash times vary between samples, a 3–5 minute minimum is common practice. Further, the sample trap acts as an additional particulate filter immediately prior to column introduction, significantly reducing clog formation and extending column lifetime.

Achieving maximum product performance and longevity

Concentrating and desalting the analyte by sample trap injection provide noticeable differences in chromtographic peak shape and resolution. Figure 1A displays the chromatogram of a bovine serum albumen (BSA) sample injected via a sample loop without a trap; Figure 1B displays the same BSA sample injected onto a sample loop with a sample trap. Methanol was employed as an organic modifier in both experiments[1].

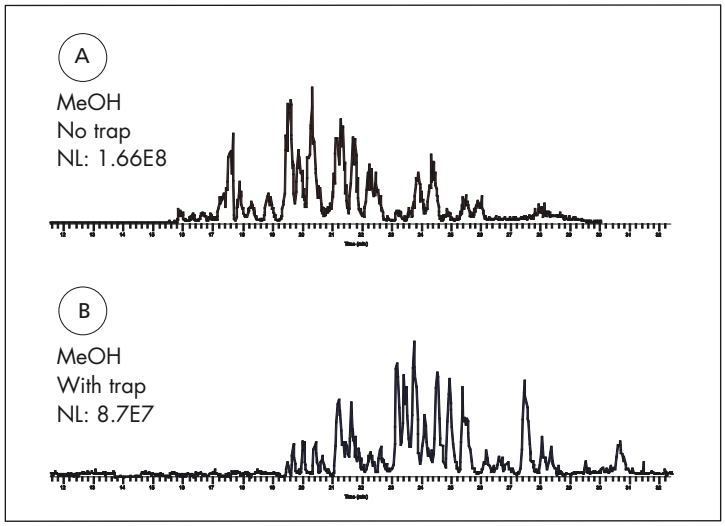

FIGURE 1

When coupled with the inline microfilter and nanofilter recommended in Tech Note PF-4 “Spray Optimization”, sample trap injections can prevent the formation of clogs and dramatically extend the service time of the nanobore LC column. Figure 2 displays nanobore LC-MS data collected after two days (Figure 2A - Injection #2) and after months of continuous operation (Figure 2B - Injection #1630).

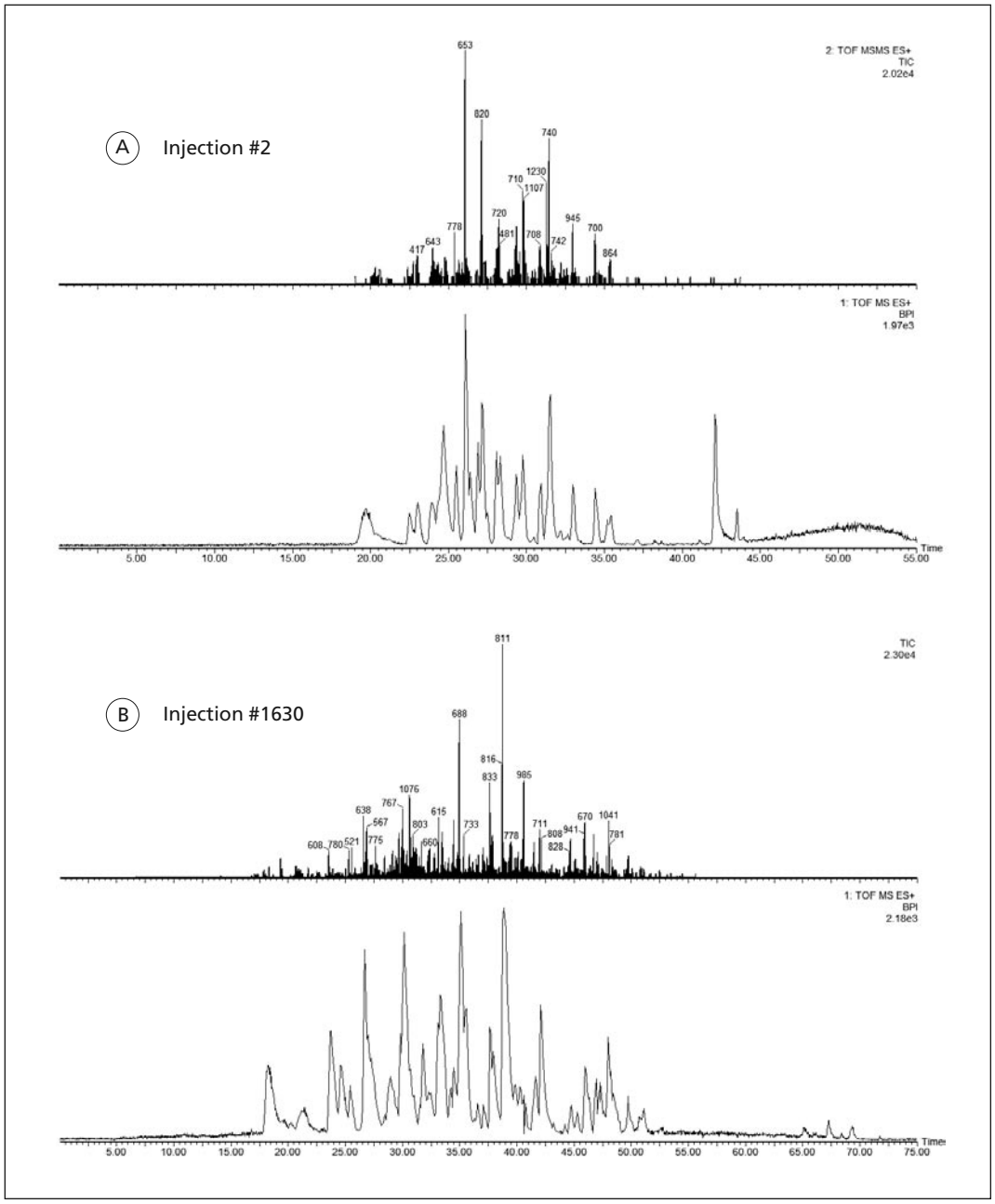

FIGURE 2 *LC-MS data collected over a period of several months with a single PicoFrit® column*

The data were collected with a 75 µm x 4 µm x 10 cm C18 PicoFrit® column with an inline nanofilter, microfilter, and 1 mm C18 trap cartridge on a Micromass® Q-Tof™ mass spectrometer. Figure 3 displays the complete configuration used in data acquisition.

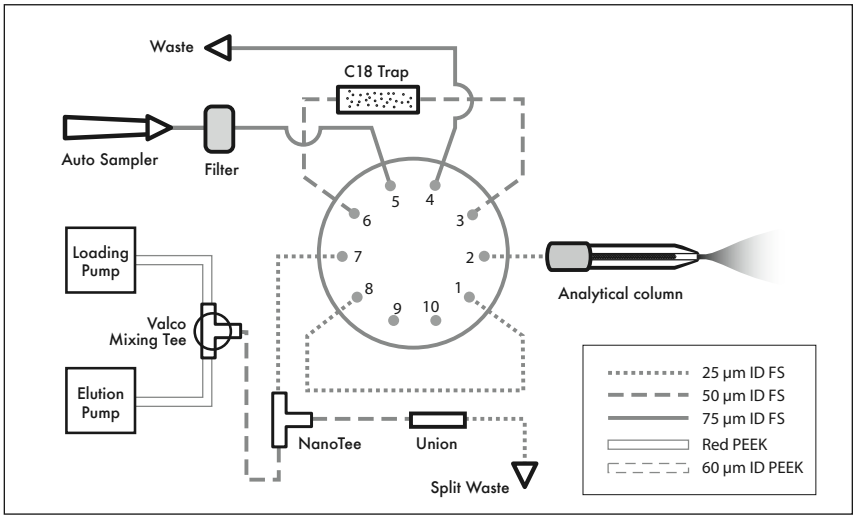

FIGURE 3 *Nanobore LC-MS configuration for data collected in Figure 2*

Procedure for sample trap injection

The following steps illustrate the loading, washing, and injection procedure using a sample trap. For this task, a 10-port valve is assumed; Positions 1 and 2 are the *load* and *inject* settings, respectively.

1) With the valve configured in Position 1, load the sample into the sample loop (Figure 4).
2) Switch the valve to Position 2. High flow rate carries the sample from the sample loop to the sample trap for washing, desalting, and analyte concentration (Figure 5).
3) After running the the mobile phase through the sample trap for several minutes, restore the valve to Position 1 (Figure 6).
4) Activate the LC gradient to introduce the analyte onto the column from the sample trap. The electrospray emitter introduces the sample into the MS inlet for analysis.

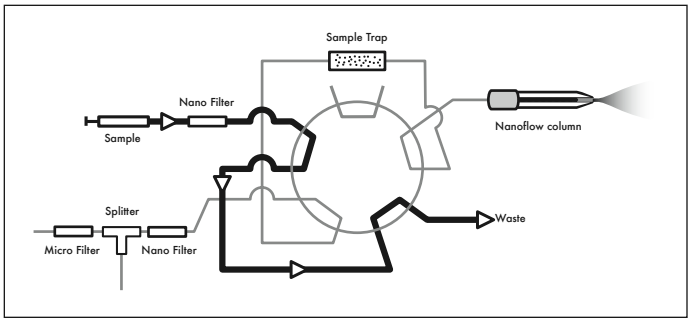

FIGURE 4 *Loading the sample loop (valve in Position 1)*

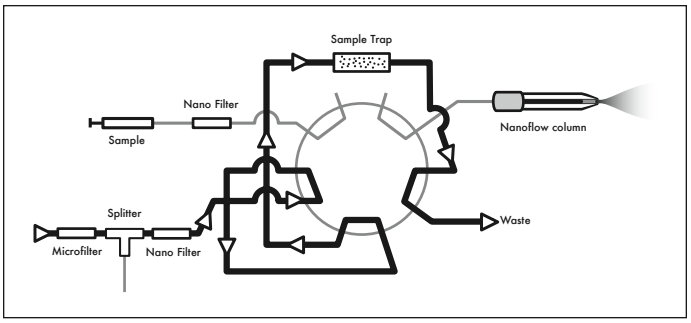

FIGURE 5 *Loading sample onto trap for concentration and desalting (valve in Position 2)*

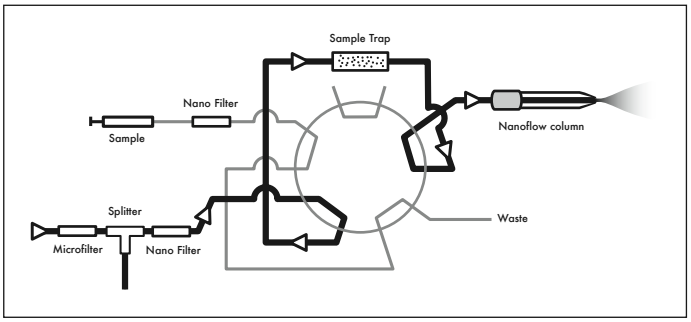

FIGURE 6 *Loading sample onto column for analysis (valve in Position 1)*

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1. Perala, A.W.; Toher, C.J.; Valaskovic, G.A. "Enhanced Nanobore LC-MS Using Methanol Gradient Elution With Peptide Mixture." Poster presented at The American Society for Mass Spectrometry, San Antonio, Texas, 2005