Improved Protein Identification by NanoLC/MS/MS using Chip-Based Columns with Integrated Post-Column Addition of DMSO for Increased Sensitivity

Jenny Albanese[2]; Christie Hunter[2]; Mark Yang[1] and Remco Van Soest[1]
[1]Eksigent, part of AB SCIEX; [2]AB SCIEX

ABSTRACT

In this poster we describe the effect on Protein identification of adding DMSO post-column in nanoLC/MS/MS

INTRODUCTION

NanoLC/MS/MS is the current method of choice for high sensitivity identification of proteins, e.g. for the discovery of biomarkers in plasma or other biological fluids. The solvent system typically used is determined by the requirements for both the chromatography and the electrospray processes. While it has been demonstrated that solvents like DMSO can increase electrospray ionization efficiency and improve sensitivity[1], the potential adverse effect on chromatographic separation has prevented researchers from adding these to the mobile phases. Post-column addition of these solvents is a good alternative, but not easy to achieve in nanoLC because of the unavoidable introduction of dispersion using a post-column Tee and additional connections. We have started to study the effect on enhanced sensitivity for protein identification using yeast samples with the DMSO post column approach.

MATERIALS AND METHODS

Sample Preparation

A 25 fmol/µl beta-Galactosidase (BG) tryptic digest solution (AB SCIEX, Part Number 4368624) was fresh prepared using 98% water / 2% acetonitrile / 0.1% formic acid as the dilution solvent. We monitored 25fmol on column of BG for each LC run. 200 ng of a yeast sample (http://www.nist.gov/), digested with trypsin, was injected on column for studying the effect of DMSO on protein identification.

HPLC

An Eksigent nanoLC 425 (AB SCIEX, Redwood City, CA, USA) was used in combination with a modified Eksigent cHiPLC® nanoflex system (AB SCIEX) in trap elute mode. Mobile phase A was water with 0.1% formic acid and B was acetonitrile with 0.1% formic acid. The gradient was 5-30% B in 15min for BG samples and 30 min for the yeast samples. Channel 1 of the 425 system was used to deliver 60 nL/min of 20% DMSO into the T-chip to get a final 5% DMSO concentration (and 10% methanol) at the spray tip. All separations were run at 300 nL/min. The Eksigent chip column is packed with 3 µm ChromXP C18 120A particles (AB SCIEX). Integrated T- chips were fabricated using standard multilayer photolithography.
The chip design has 2 components: a separation channel packed with reverse phase particles, and a side channel which intersects with the separation channel post-column for the addition of DMSO containing solvents. These chips are retrofitted into the modified Eksigent cHiPLC® system (AB SCIEX).

MS/MS and Data Processing

The MS analysis was performed on a TripleTOF® 5600+ system (AB SCIEX) equipped with a NanoSpray® Source. Data collection was done using both data dependent and data independent targeted acquisition strategies.
Protein identification data were processed using ProteinPilot™ Software. False discovery rate (FDR) analysis of yeast data was compared using a Protein Alignment Template and a ProteinPilot™ Descriptive Statistics Template.

RESULTS

Minimal change in peak width post-column addition of DMSO using T-Chip

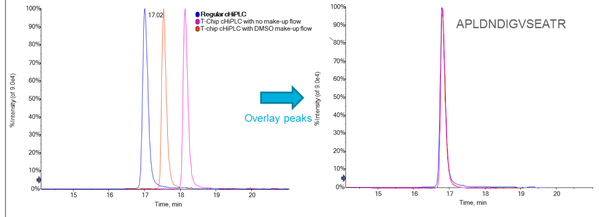

Figure 1. The overlay of one BG peptide shows three peaks acquired under different set up conditions. The blue peak represents a normal setup with out the T-chip addition. The pink peak represents a setup using the T-chip without make up flow. Finally the orange peak was observed using the T-chip with 5% DMSO makeup flow. Below are two more examples for two different BG peptides (right side). The peak height is normalized.

Gain in peak area using different % of DMSO post column

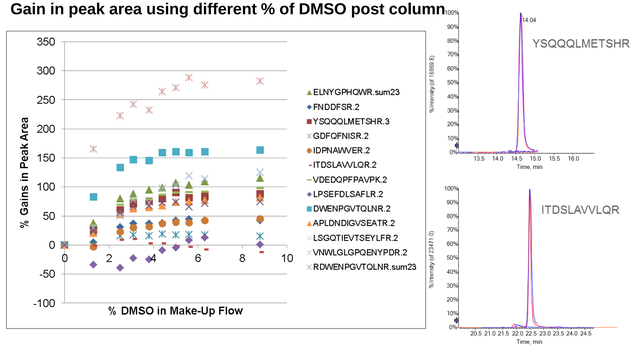

Figure 2. This graph shows the % gain in peak area for 13 different BG peptides at different DMSO percentages. This curves flattens around 5% DMSO. Certain peptides tend to ionize significantly better at higher % of DMSO. Zero point is T-Chip with no make-up flow.

Peak Area Assessment using 5% of DMSO post column

BG Peptides	Charge State	Retention Time (min)	% Gain with DMSO
ELNYGPHQWR	sum 2+3	17.4	25
FNDDFSR	2	16.6	42
YSQQQLMETSHR	3	14.9	91
RDWENPGVTQLNR	sum 2+3	17.5	74
GDFQFNISR	2	17.6	18
IDPNAWVER	2	38.9	39
ITDSLAVVLQR	2	22.8	-1.7
VDEDQPFPAVPK	2	19.5	91
LPSEFDLSAFLR	2	25.6	4.8
DWENPGVTQLNR	2	19.5	160
APLDNDIGVSEATR	2	17.9	74
LSGQTIEVTSEYLFR	2	25.2	102
VNWLGLGPQENYPDR	2	24.4	271
Median			74

Table 1. This table shows roughly a 75% gain in peak area with 5% DMSO. We observed no dependence on RT .

Protein ID of 200 ng yeast digest at different % DMSO

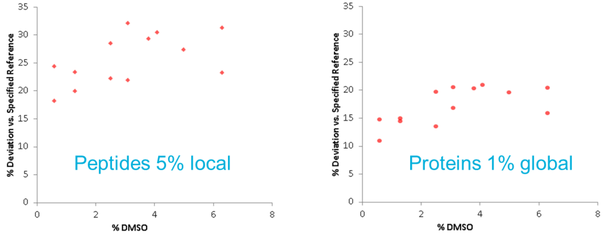

Figure 3. This graph shows a titration of DMSO % versus the % deviation of a 5% local FDR analysis on the peptide level and a 1 % global FDR analysis on the protein level. We observed a roughly 30% gain in peptides and a 20% gain in proteins, which agrees with findings described in reference 1.

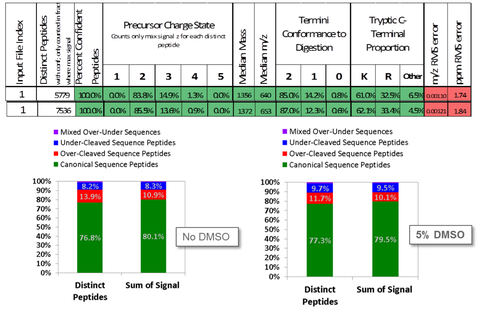

Figure 4. Peptide distribution of a tryptic yeast digest without DMSO (first row) and with 5% DMSO (second row) post column flow. The addition of DMSO shows a minimal impact on the distribution of peptides observed overall.

CONCLUSIONS

We have demonstrated that using the chips with integrated Tee, it is possible to add solvents post-column without causing additional chromatographic dispersion. We have investigated the effect of adding DMSO post-column on peptide sensitivity and the overall number of proteins identified in a complex mixture, using a QTOF type mass spectrometer. The optimal %DMSO after addition was found to be 5%, consistent with previous observations in the literature. An increase in peak area for thirteen BG tryptic peptides was observed that varied from 1 to 2.6x, with a median of 1.9x. A significant increase in proteins identified, up to 20%, has been achieved. We believe that this approach is a simple, but powerful approach to significantly improve the number of proteins identified in complex samples using nanoLC/MS/MS, benefiting biomarker discovery research. Because the ionization enhancement step is decoupled from the chromatographic step, a much broader range of solvents can now be investigated for their impact on improving nano-ESI and therefore protein identification. We are currently studying the effect of mixing DMSO with the mobile phase to compare with the post column approach.

REFERENCES

1 Bernhard Kuster, Nature Methods | VOL.10 NO.10 | OCTOBER 2013 | 989-992

TRADEMARKS/LICENSING