Effect of Supercharging Reagents in Protein Identification

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INTRODUCTION

Low abundant proteins identification and characterization using Electrospray ionization mass spectrometry (ESI-MS) in complex protein sample is always a challenge. To overcome these challenges additional supplement of supercharging agent in the same mobile phase that improves the ionization efficiency is very well reported with nano flow rate. As a result of this significant improvement in terms of response has been observed in the past. Typically, the ionization efficiency in ESI-MS is achieved by adding a small amount of supercharging reagent to analyte solution. These most commonly employed supercharging reagents are m-nitro benzyl alcohol (m-NBA) and dimethyl sulfoxide (DMSO). In the current experiment we standardized the best concentration of these above reagents in higher flow rate with complex protein tryptic digest. DMSO and m- NBA were used in different concentrations along with 0.1% formic acid to see the effect of supercharging/ Multiple charged ions on ESI-MS that allows the identification, characterization and structural analysis of peptides and large intact biomolecules. Supercharging of biomolecules (peptides) increased response of the ions as observed in ESI-MS. Furthermore, upraised signal for selected precursor ions increases efficiency of MS/MS. In the current study different concentration of DMSO (0.25%, 0.5%, 1.%, 2% and 5) were used to see elevated response of peptides with single purified protein as well as complex matrix with higher flow rate.

MATERIALS AND METHODS

Sample Preparation:

Tryptic digest of Beta galactosidase (SCIEX, Part Number 4368624 USA), Casein (Sigma, USA), Total protein digest from E. Coli and Pneumonia klebsiella were used. DMSO (Sigma, USA) and m-NBA (Sigma, USA) in various concentrations were used to get best response from Beta galactosidase digest and above organism digest. Complex protein digest was injected on CSH C18, 2.1x150 mm, 1.8 µm column with a 60-min linear water – acetonitrile gradient at 300 µl/min flow rate, where data was acquired in information dependent acquisition (IDA) mode in SCIEX TripleTOF® 6600 system.

In other independent experiments out of various concentration of DMSO, (0.25%, 0.5%, 1.%, 2% and 5) 1% was selected in combination with 0.025% (V/V) m-NBA to see combined effect in mobile phases.

HPLC Conditions:

ExionAD™ UHPLC system (SCIEX) was used to separate the digested protein on Phenomenex CSH C18, 2.1x150 mm, 1.8 µm column . Standard Mobile phase A was water with 0.1% formic and B was acetonitrile with 0.1% formic acid. The gradient was 5-50% B in 50min with total run time 60 minutes at flow rate of 300ul /min .Various concentrations (0.25%, 0.5%, 1.%, 2% and 5) of DMSO and m-NBA was separately added into the mobile phases.

MS/MS Conditions:

The MS analysis was performed on a TripleTOF® 6600 system (SCIEX). Data collection was done using data dependent acquisition strategies with following parameters, TOF MS m/z range of 350-2000 and MSMS m/z range of 100-1800. Protein identification data was processed using ProteinPilot™ Software 5.0. Extract Ion chromatogram(XIC) was processed using PeakView™ Software 2.2.

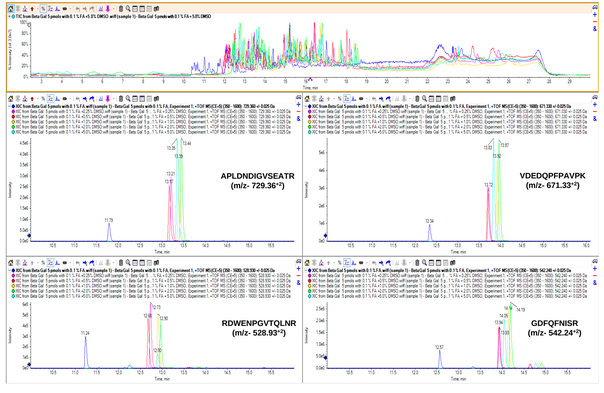

Figure 1. Effect of supercharging agent (DMSO) used in various concentration , (0.25%, 0.5%, 1.%, 2% and 5) on peptide response

Experiments	Time (min)	Area (m/z-671.33)	Fold Change
0.1% FA	12.32	3129343.97	
0.1% FA +0.25%DMSO	13.72	10375783.78	3.32
0.1% FA +0.5%DMSO	13.98	9857607.89	3.15
0.1% FA +10.%DMSO	13.90	14004822.00	4.48
0.1% FA +2.0%DMSO	13.95	18184001.35	5.81
0.1% FA +5.0%DMSO	13.81	15426646.84	4.93
Experiments	Time (min)	Area (m/z-729.36)	Fold Change
0.1% FA	11.76	2973318.83	
0.1% FA +0.25%DMSO	13.19	9928141.28	3.34
0.1% FA +0.5%DMSO	13.45	9298425.02	3.13
0.1% FA +1.0%DMSO	13.37	13746889.97	4.62
0.1% FA +2.0%DMSO	13.43	17797745.21	5.99
0.1% FA +5.0%DMSO	13.32	16710653.90	5.62
Experiments	Time (min)	Area (m/z-582.93)	Fold Change
0.1% FA	11.22	935048.89	
0.1% FA +0.25%DMSO	12.70	1828635.19	1.96
0.1% FA +0.5%DMSO	12.97	1424139.26	1.52
0.1% FA +1.0%DMSO	12.88	1740935.17	1.86
0.1% FA +2.0%DMSO	12.95	1283042.83	1.37
0.1% FA +5.0%DMSO	12.87	483786.42	0.52
Experiments	Time (min)	Area (m/z-542.24)	Fold Change
0.1% FA	12.54	1798385.81	
0.1% FA +0.25%DMSO	13.94	5919980.03	3.29
0.1% FA +0.5%DMSO	14.21	5250454.11	2.92
0.1% FA +1.0%DMSO	14.13	7722035.66	4.29
0.1% FA +2.0%DMSO	14.19	7673751.21	4.27
0.1% FA +5.0%DMSO	14.02	5945389.51	3.31

Table 1. Fold change of various peptides In presence of different concentration of DMSO. 1% DMSO is the most effective and consistent for all peptides.

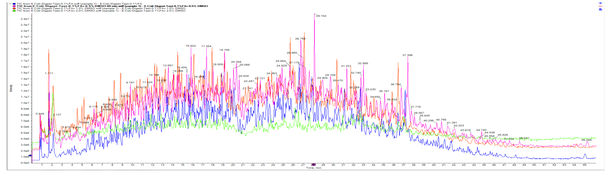

Figure 2. TIC overlay of *E. Coli* protein Digest at different concentration of DMSO

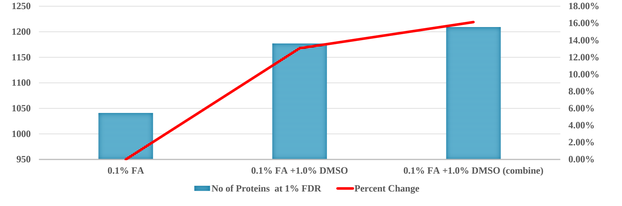

Figure 3 . No of protein identified in *E. Coli* at 1% FDR and respective fold change (Control vs DMSO)

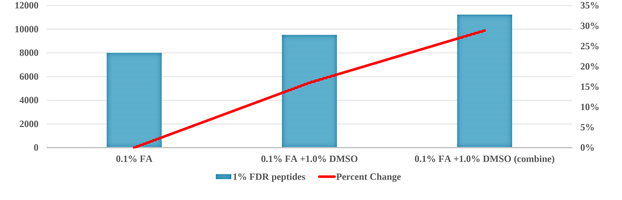

Figure 4 . No of total peptides identified in *E. Coli* at 1% FDR and respective fold change (Control vs DMSO)

RESULTS

We report an average 2-3 times fold change in the intensity with respective peptides in single protein and over all more than 16-20% additional proteins were identified in complex protein digest from ***Escherichia coli*** and ***Klebsiella Pneumonia*** respectively.

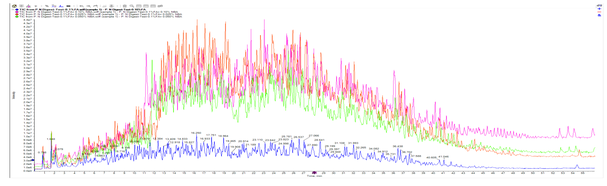

Figure 5. TIC Intensity variance of all peptides in the presence of different concentration of m-NBA

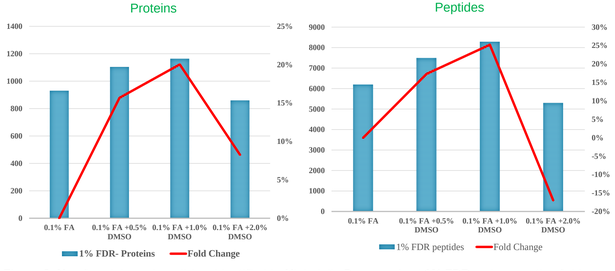

Figure 6: No of protein and peptides identified in *Klebsiella Pneumonia* at 1% FDR and respective fold change

CONCLUSIONS

- The concentration of DMSO in the mobile phase to achieve best sensitivity was optimized and was 1% (v/v) at higher flow rate for proteins peptides.
- Addition of 1% (v/v) DMSO in the mobile phase did not alter the retention behavior of peptides.
- Addition of 1% (v/v) DMSO has improved overall sensitivity of peptides of various proteins.
- Significant improvement in the number of proteins identified in complex samples using UHPLC was observed, benefiting biomarker discovery research.

REFERENCES

1. Harry.J et all, Protein Conformation and supercharging with DMSO from aqueous solution. J.Am.Soc Mass Spectrum 22: 1178-1186 (2011)
2. Hannes et all, DMSO enhances electrospray response, boosting sensitivity of proteomic experiments, Nature Methods 10, 989–991 (2013)

TRADEMARKS/LICENSING

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