Optimizing Digestion Efficiency: Impact of Various Denaturing Reagents on a 60-Biomarker Health Surveillance Panel in Human Plasma

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Introduction

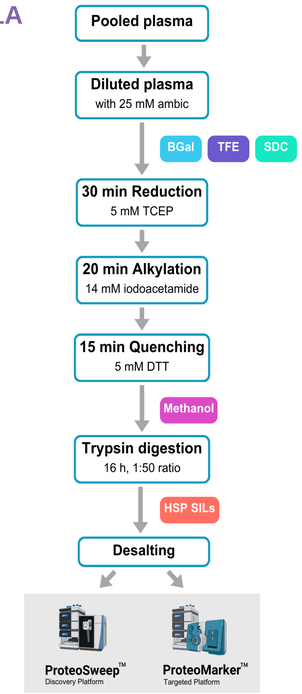
The efficiency of sample preparation protocols holds paramount significance in plasma biomarker discovery and targeted mass spectrometry analysis. The aim of this study is to assess and compare the efficacy of various denaturing reagents in plasma protein digestion. Employing a comprehensive 60-biomarker health surveillance panel (HSP) and utilizing both discovery and targeted mass spectrometry methods, our findings underscore a noteworthy advancement in denaturing reagent efficacy. The outcome of this investigation offers valuable insights into elevating endogenous peptide intensities, providing a better understanding of the optimal conditions for sample preparation in clinical proteomics.

Methods

Five denaturing reagents were evaluated for sample preparation (Fig 1A): TFE (50%) as the solvent, SDC (5%) as the surfactant, a combination of SDC (5%) and methanol (20%), a combination of TFE (50%) and methanol (20%), and methanol (20%) as the solvent (Fig 1B) . To ensure robustness, each condition was replicated five times within 96-well plates. The digestion protocol was scrutinized through the quantification of 60 plasma proteins (HSP), incorporating b-galactosidase (BGal) as an internal standard with spiked-in SILs. Sample analysis was done both on Sciex QQQ (targeted MRM method) and an Orbitrap Exploris (discovery DIA and DDA methods) to evaluate trypsin digestion effectiveness and the number of detected proteins/peptides. Data analysis was performed using Precision Biomarker Laboratories' proprietary platform ProEpic™, Proteome Discoverer with Chymeris, and Skyline.

Figures

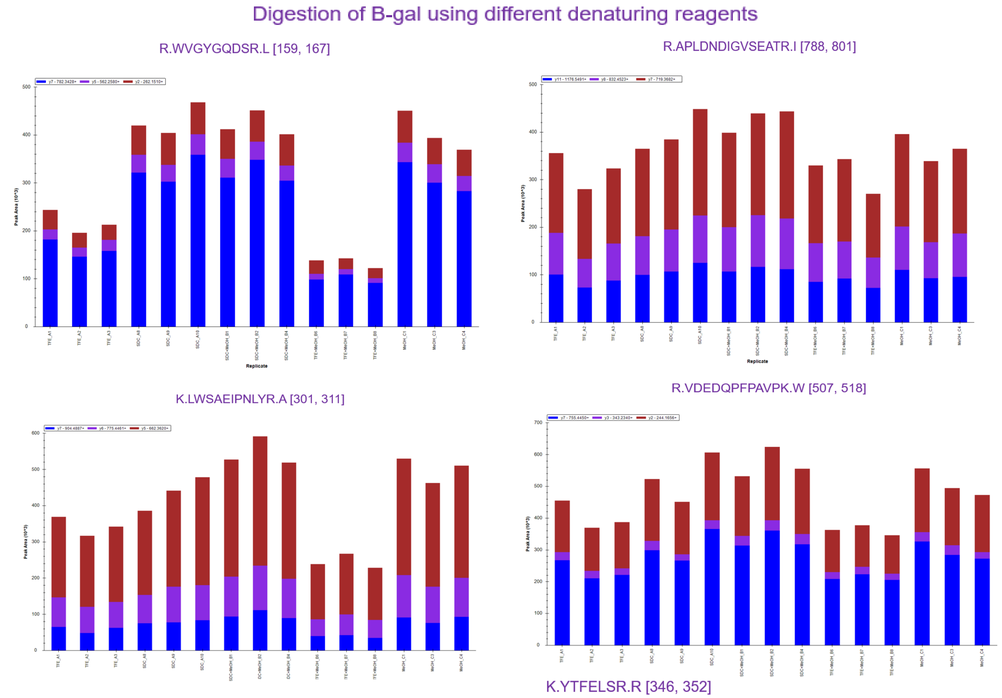
1A



1B

Denaturation agents		Denaturing conc.	Digestion conc.
solvent	TFE	50% v/v	5% v/v
surfactant	SDC	5% w/v	1% w/v
solvent	MeOH	20% v/v	20% v/v
combination	TFE MeOH	50% v/v 20% v/v	5% w/v 20% v/v
combination	SDC MeOH	5% w/v 5% w/v	1% w/v 20% v/v

2



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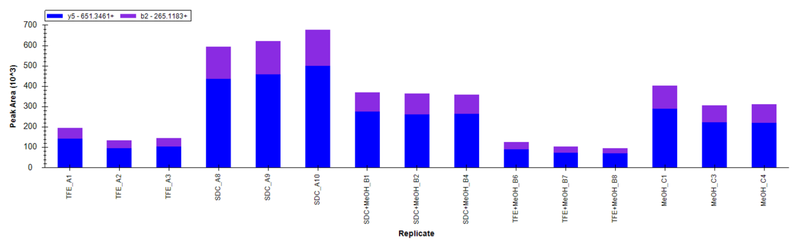
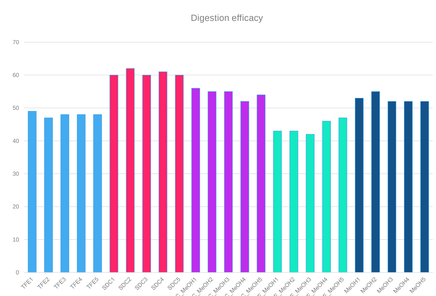


4A



4B

Sample	Digestion Efficacy (avg.)	Standard Deviation
TFE	48	0.63
SDC	61	0.80
MeOH	54	1.36
TFE MeOH	44	1.94
SDC MeOH	53	1.17

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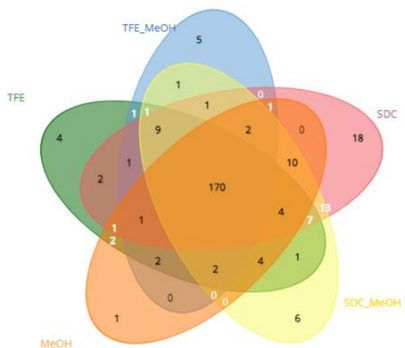

Results

Targeted Analysis

2. MRM data analysis using Skyline (v23.1.0.268) revealed notable differences in peak intensities of BGal peptides based on denaturing reagents.

2. BGal peptides exhibited significantly higher peak intensities with SDC (and SDC + methanol) compared to TFE and TFE + methanol.

2. Methanol yielded robust and high peaks comparable to those achieved with SDC.

3. SDC demonstrated the highest signal within a comprehensive 60-biomarker HSP.

Discovery Analysis

4A. DIA data analysis with ProEpic™ (Thermo DIANN 1.8.1 search pipeline) showed varying digestion efficacy among denaturing reagents.

4B. SDC demonstrated the highest digestion efficacy at 61%, followed by SDC + methanol and methanol alone, both at 53%.

4B. TFE exhibited a digestion efficiency of 48%, while TFE + methanol showed slightly lower efficiency at 44%.

5. In terms of total protein and peptide detection, analysis using Proteome Discoverer with Chymeris (v3.1) highlighted the superiority of SDC over TFE.

Conclusions

Our study highlights the crucial role of denaturing reagents in optimizing sample preparation protocols for clinical proteomics. SDC emerged as the most effective denaturing agent in our assay, exhibiting superior peak intensities and digestion efficacy compared to other reagents. These findings underscore the significance of selecting appropriate denaturing reagents to enhance data quality and reliability, emphasizing the importance of sample preparation in achieving accurate and reproducible results in clinical proteomics.

Acknowledgements/Conflict of Interest

The authors declare no competing financial interest.