Proteomic study of decidua in recurrent pregnancy loss patients.

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Introduction

Those assays must be resistant to working circumstances. Proteomic biomarkers will be difficult to translate into the clinic without reliable assays. This paper describes a strategy for identifying a reliable operating window for proteomics assays and applies it to Sequential Window Acquisition of All Theoretical Spectra Mass Spectroscopy. We employed a sequential quality by design strategy to find essential SWATHMS parameters using a fractional screening design, then used response surface methods to determine a robust operating window with good reproducibility, and finally validated those settings in a separate validation study. Two crucial SWATHMS parameters were discovered throughout the screening process. We calculated the number of proteins and repeatability as a function of those factors, resulting in a stable operating window for the amount of proteins measured in human serum. These settings were found to provide strong proteome broad coverage and high quantification reproducibility in a separate validation investigation. Design of experiments can be used to find a stable operating window for SWATH MS. The technique allows for a better knowledge of proteomics assays as well as increased data-driven confidence in SWATHMS performance [1].

It is not enough to optimise proteomics assays in translational medicine. Those tests must be resistant to operational circumstances and easily transfer to other devices in other laboratories. Proteomic biomarkers will be difficult to translate into the clinic without reliable assays. Because it can represent the physiological changes associated with disease, the human blood proteome is one of the more therapeutically relevant matrices for biomarker identification. The fact that it transports not just blood proteins but also other proteins such as messengers between tissues or tissue damage products adds to its relevance and complexity [1].

By increasing the signal-to-noise ratio of observed transitions and, as a result, the number of peptides and/or proteins per sample that can be identified and/or quantified, robust optimization of sample preparation, LC, and SWATH acquisition parameters may improve results in the analysis of complex protein samples [2]. Using SWATH-MS parameters adjusted from the original description, different investigations have shown an increase in both the quantity of proteins discovered and quantification reproducibility [3]. However, these investigations found that the parameters that influence SWATH-MS detection are highly dependent on both the hardware and the material being analysed, and hence should be evaluated empirically for various scenarios. The goal of this work was to increase the amount of proteins and peptides measured per SWATH analysis on human serum samples without sacrificing precision [4]. To accomplish so, we looked at the impact of several parameters and their interactions, such as the initial sample input, the length of the LC separation, and various MS acquisition settings. We used a DoE approach, which is entirely scalable to other samples and devices and could help other researchers save time and money by optimizing their operations. Furthermore, we conducted validation tests on raw and depleted materials using the improved approach [5].

Conclusion

In addition to two process-critical factors, a structured, planned approach to SWATH-MS development allowed the discovery of five parameters to which the method was rather resilient. By modelling the two crucial parameters, a robust operating window for the method was identified, maximizing both the number of proteins measured and the method's reproducibility. Prior to a big consortium investigation with several partners, the resulting settings provided a high level of confidence in the procedure.

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