INCREASED THROUGHPUT FOR 2D LC IN THE ANALYSIS OF HUMAN PLACENTAL SAMPLES

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Martha Stapels[1], Keith Fadgen[1], J. Will Thompson[2], M. Arthur Moseley[2], and James Langridge[1]
[1]Waters Corporation, Milford, MA; [2]Duke University School of Medicine, Durham, MA

INTRODUCTION

Most proteomic samples, when digested, generate peptides with similar distributions of hydrophobicities (1). The complexity of these samples requires orthogonal methods of separation (in LC or MS) to identify and quantify all detectable peptides in a sample. Changing the pH between 10 and 2.6 has been shown to dramatically change the selectivity of a reverse-phase separation (2). The need for multiple dimensions of separation must be balanced by the analysis time for each sample. For this reason, a high throughput 2D RP/RP method was developed and used in combination with ion mobility to analyze peptides from human placental samples.

METHODS

LC/MS: nanoACQUITY UPLC® with 2D Technology SYNAPT™ G2-S HDMS™

Sample Preparation: Proteins were reduced, alkylated, and digested in-solution with trypsin.

First Dimension:

Column: 300 µm x 5 cm XBridge™ C_{18} (5 µm)
Gradient formation: discontinuous step gradient
Eluent A: 20 mM ammonium formate pH 10.0
Eluent B: acetonitrile

Second Dimension:

Trap column: 180 µm x 2 cm Symmetry C18 (5 µm)
Column: 75 µm x 15 cm HSS T3 C_{18} (1.8 µm)
Eluent A: 0.1% formic acid in water
Eluent B: 0.1% formic acid in acetonitrile

Online Dilution with RP/RP: To maximize sample recovery on the second dimension trap column from the organic-containing fractions, an aqueous flow was delivered with the 2nd dimension pump, and mixed with the eluted fraction prior to trapping.

MS Data processing : MS^E data were processed and searched with ProteinLynx Global Server (PLGS 2.5.2) with Identity^E informatics.

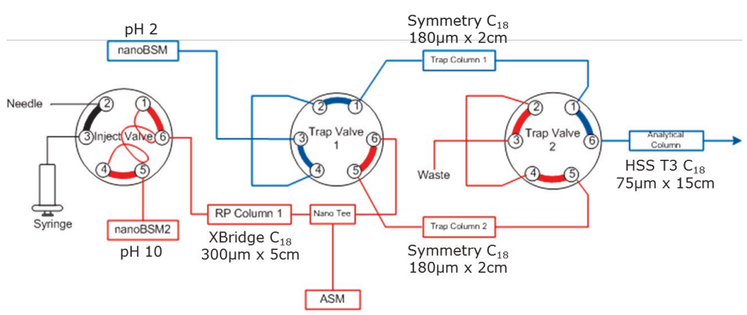

Figure 1. Fluidic layout for the high throughput RP/RP analysis with online dilution. Loading on one trap column takes place during elution of the other.

RESULTS

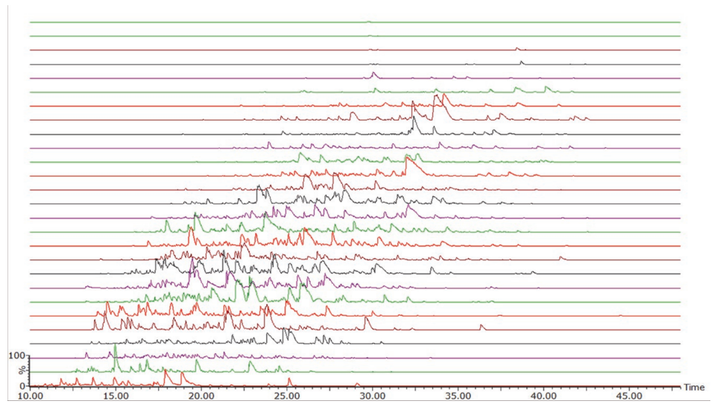

Figure 2. BPI Chromatograms for the 28 fraction experiment used for optimization.

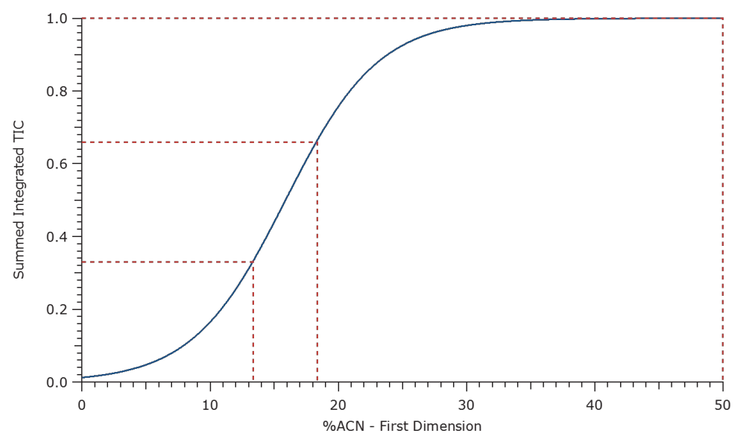

Figure 3. Summed integrated TIC for the 28 fraction experiment. The cuts for a 3 fraction experiment are highlighted.

In order to determine optimum fractionation with the high-low pH RP/RP system, a 28 fraction experiment was performed to map out the separation space. First dimension cuts of 5, 7, 8-28 in 1% steps, 30, 32, 35, 38, and 50% acetonitrile were chosen to properly interrogate the separation space. Chromatograms for each fraction are in Figure 2.

For each fraction, the total ion chromatogram (TIC) was integrated across its entire separation space. The results were summed, normalized, and plotted against the percent ACN used for each cut. Figure 3 shows the optimum fractionation strategy for the RP/RP separation space. As an example. the cuts for a 3 fraction experiment are depicted.

Using a total analysis time of 4 hours, human placental samples were run with 3 or 6 fractions in the first dimension. Chromatograms from the method using 6 fractions are shown in Figure 4. As shown in Figure 5, the 6 fraction method consistently yielded more identifications.

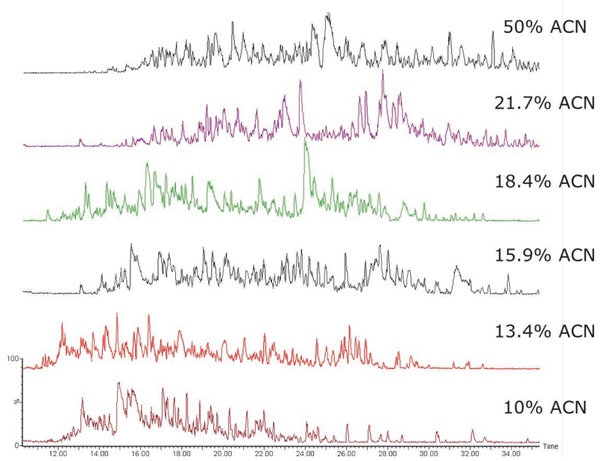

Figure 4. Example 6 fraction results for human placenta samples in 4 hours.

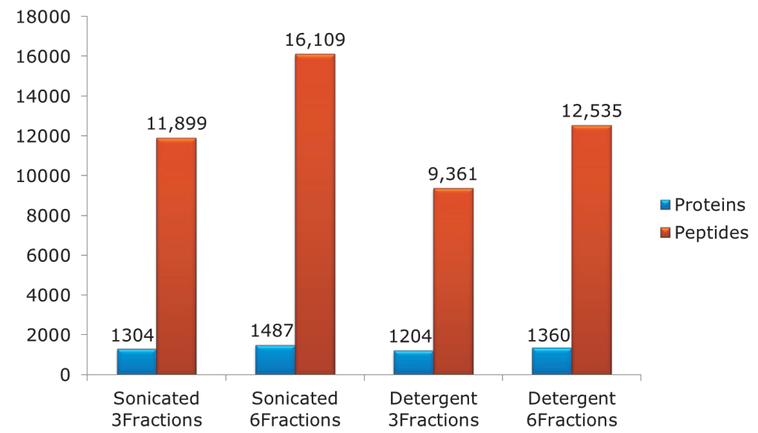

Figure 5. Number of identified proteins and peptides from both a sonicated and detergent extracted placental sample, using either a 3 or 6 fraction 2D method over 4 hours.

CONCLUSION

- **Optimized prediction strategy for high/low pH RP/RP separations**
- **With high throughput 2D, more proteins identified per hour**
- **>90% of peptides observed in only a single fraction**
- **Increasing peak capacity with orthogonal methods beneficial for identifying and quantifying proteins in complex mixtures**

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Monique Chireau of Duke University for the human placenta samples used in this study.

REFERENCES

1. Geromanos S. et. al, Proteomics. 2011, 11, 1189-211
2. Gilar M. et. al, J. Sep. Sci. 2005, 28, 1694-703

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS