A Novel Phenyl-Based RPLC Stationary Phase for High Throughput, High Resolution Characterization of Protein Therapeutics

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Protein therapeutics can be effectively characterized using the capabilities of liquid chromatography (LC). Because of its high resolving power and amenability to mass spectrometric (MS) detection, reversed phase liquid chromatography (RPLC) has become one of the most heavily relied upon techniques. However, RPLC stationary phases notoriously suffer from performance limitations, including their strong dependence on ion pairing and elevated separation temperatures that can cause on-column degradation.

To address these limitations, a novel column technology has been designed. This column technology is based on an optimized 2.7 µm superficially porous particle that by van Deemter analyses has proven to be effective in minimizing intra-particle diffusion, thereby affording a kinetic efficiency advantage. For the porous layer of this stationary phase, an optimal pore diameter has also been carefully selected. Comprehensive analysis of intact and IdeS-digested monoclonal antibodies (mAbs) has shown that the average pore diameter needs to be at least 400 Å, particularly when ion pairing is minimized for MS compatibility, where proteins are more likely to adopt extended structures. Moreover, the capability of this column technology is augmented by a novel surface chemistry that is synthesized using a multistep silanization process to yield a phenyl-based bonded phase which is both high in coverage (up to 6 µmol phenyl moiety/m^2) and comprised of rigidly constrained carbons. This novel bonded phase is believed to limit silanol interactions by extensively masking the silica base particle, to facilitate more discrete desorption at lower temperatures by minimizing the conformational heterogeneity of protein adsorption, and to improve resolving power by being highly retentive. Using either HPLC or UHPLC instrumentation, it will be shown that this technology has made it possible to better characterize mAb and ADC therapeutics by delivering unprecedented resolution as well as higher fidelity, higher quality data.

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Matthew Lauber is a principal scientist within Chemistry R&D at Waters, where he leads an evaluation team that focuses on developing new consumables for the analysis of biomolecules. Since his graduate studies in proteomics at Indiana University, he has, for six years, been applying his expertise in protein chemistry and LC-MS based characterization methods toward the development and application of state-of-the-art reagents and separation chemistries. In this time, Matthew has helped introduce several new and noteworthy approaches, including glycan profiling through the use of novel labeling and sample preparation techniques, investigating hydrophilic modifications on intact proteins by wide-pore amide HILIC separations, and using charge doped C_{18} phases for higher resolution peptide mapping. Among other things, his group is now pushing towards new capabilities for protein reversed phase chromatography.

Experience

Principal Scientist **July 2012 - Present**

Waters Corporation, Chemistry R&D, Milford, MA

Development of reagent and separation technologies for the characterization of biotherapeutics:

- Charge-doped C_{18} for high peak capacity peptide separations
- High sensitivity LC-MS for host cell protein identification
- Rapid preparation and HILIC analysis of N-glycans using a fluorescent, MS-enhancing labeling reagent
- Optimization of HILIC-MS for glycopeptide characterization and profiling of glycosylation site

Graduate Research **May 2007 – June 2012**

Dr. James P. Reilly, Indiana University, Bloomington, IN

Structural proteomics through combining chemical derivatization and MS:

- Development of a novel amine-reactive cross-linking reagent
- Application of multi-dimensional separations along with advanced mass spectrometry to characterize intact proteins and peptides in complex mixtures
- Affinity chromatography, UV-Vis spectroscopy, cell culturing, and isolating macromolecular complexes by sucrose density gradient fractionation

Intern, Materials Engineering **August 2005 - May 2007**

GE Commercial Motors by Regal-Beloit, Fort Wayne, IN

- Directed quality control, conducted failure analyses, and aided in product development
- Employed analytical instrumentation (FT-IR, TGA, DSC, and SEM-EDX) to characterize materials
- Applied the statistical software JMP to analyze data according to Six Sigma DMAIC training

Education

Ph.D., Biological Chemistry **June 2012**

Indiana University, Bloomington, IN

Thesis: Combining Cross-Linking, Separations and Mass Spectrometry to Study Protein Structures and Interactions

B.Sc. (Hons), Chemistry **May 2007**

Purdue University, Fort Wayne, IN

Publications

D'Atri, V.; Fekete, S.; Beck, A.; Lauber, M. A.; Guillarme, D., Hydrophilic Interaction Chromatography Hyphenated with Mass Spectrometry: A Powerful Analytical Tool for the Comparison of Originator and Biosimilar Therapeutic Monoclonal Antibodies at the Middle-up Level of Analysis. *Analytical Chemistry* **2017,** *89* (3), 2086-2092.

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Lauber, M. A.; Reilly, J. P., Structural analysis of a prokaryotic ribosome using a novel amidinating cross-linker and mass spectrometry. *Journal of Proteome Research* **2011,** *10* (8), 3604-16.

Chang, F. M.; Lauber, M. A.; Running, W. E.; Reilly, J. P.; Giedroc, D. P., Ratiometric pulse-chase amidination mass spectrometry as a probe of biomolecular complex formation. *Analytical Chemistry* **2011,** *83* (23), 9092-9.

Lauber, M. A.; Reilly, J. P., Novel amidinating cross-linker for facilitating analyses of protein structures and interactions. *Analytical Chemistry* **2010,** *82* (18), 7736-43.

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