Microwave-assisted digestion of monoclonal antibodies

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The last decades have secured the importance of biological therapeutics, especially monoclonal antibodies (mAbs), that have shown efficacy against promising treatments for diseases such as cancer, hormone repositioning, vaccines, and many others. However, the inherent variability of these protein-based therapies demands a thorough physicochemical assessment. Since mAbs possess many crucial post-translational modifications (PTMs) for the correct protein structure and function, the multi-attribute analysis has gained attention as a new and necessary quality control for the industry. Protein sample preparation, though effective, is a limited and time-consuming multi-step laborious process encompassing at least 1 hour and a half for protein reduction and alkylation before overnight trypsin digestion (approximately 16 hours). Furthermore, in these steps, chemical artifacts can be attached to the protein structure during these processes, such as deamidation, oxidation, N-terminal pyroglutamination, glycation, and others that can alter the correct analysis of the biopharmaceutical native structure. In this context, the study and improvement of faster and simpler methodologies that interfere less with the protein structure is of great importance to the biopharmaceutical industry for reliable quality control. The use of microwaves is a common strategy to accelerate the rate of biochemical reactions, including microwave assistance to decrease the time required for enzymatic reactions. With this characteristic in view, we sought to test the use of a conventional microwave to assist trypsin digestion of monoclonal antibodies to comprehensively map the level of identified peptides containing PTMs and glycosylation profile, comparing to the most commonly applied overnight strategy. We utilized a known protein (bovine serum albumin – BSA) to validate the microwave trypsin digestion, and the best condition was also tested in commercial IgG from human serum. The single protein was reduced with DTT or TCEP at 100°C for 10 minutes and alkylated with IAA or CAA for 30 minutes at 30°C, followed by protein chloroform-methanol precipitation and a 10-minute tryptic digestion. The raw data was then analyzed with MaxQuant, allowing 4 missed cleavages. Carbamidomethyl (C) was set as fixed modification, and Oxidation (M), Acetyl (Protein N-term), GlyGly (K), Amidated (Protein C-term), Deamidation (NQ), Gln->pyro-Glu, Hex (K) as variable modifications, and dependent peptide search was also performed. All MaxQuant resulting data was analyzed with Python (pandas, numpy, matplotlib, seaborn, scipy.stats, functools), and were condensed into a single file containing all modified and unmodified peptides. This data was analyzed by comparing the z-score of all normalized intensities and counts for modifications identified in all conditions tested. Peptides were also analyzed statistically through a t-test for specific PTMs of interest. Our methodology has shown that microwave 10-minute digestion has a better sequence coverage when compared to the overnight approach, as well as a similar PTMs intensity yield, but quantitatively less modified peptides.,

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