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Simultaneous absolute quantification and structural characterization of therapeutic monoclonal antibodies after administration to patients using capillary electrophoresis-tandem mass spectrometry

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Infliximab (IFX) is a chimeric monoclonal antibody (mAbs) approved mainly for the treatment of Crohn's disease. Because IFX is administered over long-term periods for the treatment of a chronic pathology, it is important to monitor its concentration in patient serums in order to adjust the treatment if necessary. Also, the clinical practice recently pointed that some patients may show unexpected response without providing any tangible interpretation. Patients follow-up is currently performed only through quantification using ELISA immunoassay, however in some cases specificity may be problematic due to matrix effects.
We developed a novel analytical strategy based on capillary electrophoresis hyphenated to tandem mass spectrometry (CE-MS/MS) for the absolute quantification and concomitant structural characterization of IFX in human serum. A dedicated serum purification process was designed to provide optimal sensitivity and compatibility with the CE-MS/MS analysis. Purified IFX peptides obtained from tryptic digestion were separated and characterized by CE-MS/MS. CE-MS/MS method demonstrated the successful quantification of IFX in spiked serum for concentration ranging from 0.4 to 25 µg/mL. Structural characterization of IFX was performed simultaneously using the same dataset. CE-MS/MS data allowed to successfully characterize the structures of six major N-glycosylation and establish a detailed glycoprofiling of IFX in serum samples. Also, six PTMs of interest, including asparagine deamidation and aspartic acid isomerization, were precisely characterize regarding localization and modification levels. CE-MS/MS analytical strategy was applied to serum samples originating from 24 patients treated for Crohn's disease using IFX. Results exhibited an important disparity regarding the evolution of IFX concentration after administration between the different patients. Also, CE-MS/MS data demonstrated a gradual Asp57 deamidation during IFX residence time in the patient's system that was not described in the literature. This residue is located in the region of IFX directly interacting with TNF-α, and therefore the modification could alter the activity of IFX. As a consequence, the study emphasized the possibility provided by CE-MS/MS to achieve an additional dimension of characterization regarding the outcome of mAbs after administration.