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Development and validation of analytical methods based on RP-HPLC: Quantifying HER1 extracellular domain in culture supernatant and peptide mapping of a monoclonal antibody

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Techniques based on high-resolution liquid chromatography are currently used to quantify recombinant proteins from culture supernatants, as well as their characterization. Such assays can be easily and rapidly developed, this is the case of reverse phase chromatography. In this study, we describe the development and validation of an analytical technique for quantifying HER1 extracellular domain (HER1 ECD) in bioreactor supernatant using reversed-phase chromatography with a C8 column. On the other hand, we validate the methodology for the peptide mapping of monoclonal antibody using C4 column. For both study cases, the proteins were analyzed by monitoring the absorbance of the sample at 214 nm. The resulting analytical methodology was found to provide precise and accurate results for a wide range of concentrations (10–120 µg/mL) of HER1 ECD. The accuracy of the method varied from 86 to 109%, while the repeatability and the day-to-day intermediate precision were less than 7.25 and 7.85%, respectively. In the case of peptide mapping of mAb, the methodology provides a range of 35-40 well resolved peaks. As a criterion is set RT ≤0.5 min and the percentage peak height relative to the reference material must be 70% to 130%. These methodologies constitute a useful tool that can be applied during the production of the HER1 ECD vaccine and in the identification of modifications on the primary structure of the mAb due to changes in bio-manufacturing process.

Biography

Yadira S Prieto Curbelo completed her Graduation in Biochemistry in 2005 and MSc in 2010 at University of Havana, Cuba. She has expertise in different areas, such as: purification and development of analytical techniques and; in proteomic studies of expression stability in recombinant NS0 cell lines. She developed and validated chromatographic techniques for the mAbs characterization and quantification. She has expertise in mass spectrometric for characterization of proteins and oligosaccharide residues. She has been serving as Reviewer of Cytotechnology journal and member of Cuban Society of Pharmacy.

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