B9 - EVALUATION OF A CANDIDATE NATIONAL STANDARD FOR RECOMBINANT HUMAN ERYTHROPOIETIN BY UPLC/HDMS^{E}

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Objective: Recombinant human Erythropoietin (rh-EPO), a 34 kDa glycoprotein, is the most used biopharmaceuticals in the world. In this work, from a candidate national standard developed for rh-EPO a methodology was established to evaluate impurities and to elucidate the structure of peptides in protein digests by UPLC/HDMS^{E}.

Methods: Peptide mapping and intact protein analysis were performed. For peptide mapping specificity and selectivity were increased based on the active ion mobility separation by T-wave device inside the mass spectrometer in positive ion mode with electrospray ionization and UPLC configured with HSS T3 column, flow rate: 800 uL/min, column temperature: 60˚C mobile phase A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile. The batch was digested trypsin after reduction and alkylation. Reduced protein was injected using Massprep Intact protein analysis kit. For intact protein analysis was performed using micro desalting column and 1,5 ug injected. The data was processed using Biopharmalynx software to confirm the protein sequence.

Results: The raw spectrum of rh-EPO tryptic peptides was processed and 99,4 % rh-EPO sequence was verified. The deconvoluted mass spectrum is showed for intact protein analysis and information on heterogeneity was measured. The glycosylation sites were also identified in N-linked N24, N38 and N83 and O-linked S126 which is in agreement with the literature.

Conclusion: It was demonstrated that candidate could be used as national standard for rh-EPO final product and peptide mapping identification. The characterization of rh-EPO peptide maps was showed with high sequence coverage and was successful identified in

the batch. The results clearly show the benefits in terms of software and data analysis by mass spectrometry and UPLC to sequence confirmation and post translational modification.