B12 - IDENTIFICATION AND QUANTIFICATION OF SOMATHROPIN MODIFICATIONS BY PEPTIDE MAPPING WITH UPLC/HDMS^{E}

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Objective: Somathropin is part of an important class of biopharmaceutical product. This product is subject to a variety of covalent modifications that could be occur during manufacturing, formulation and storage. In this work was developed a new methodology for identification and quantification of covalent modifications, by UPLC/HDMS^{E}.

Methods: We developed a methodology based peptide mapping for detection of covalent modifications and characterization of intact somathropin without enzymatic degradation. The somathropin sample and standard were digested with enzyme trypsin after reduction with dithiothreitol and alkylation with iodocetamide. The resulting peptide mixture was separated using ultra performance liquid chromatography (UPLC) system using C_{18} column, with a mobile phase A 0,1% formic acid in water and mobile phase B 0,1% formic acid in acetonitrile. A gradient elution was employed with flow was set at 600µL/min. and detection using high definition mass spectrometry (HDMS) with electrospray ionization on a UPLC/HDMS^{E} system. Freshly prepared tryptic digest (10 pmol) was injected for the UPLC/HDMS^{E} analysis. The analysis was repeated three times. The acquired data were processed by Biopharmalynx Software. The processed data were first searched against a somathropin database with trypsin specificity and one potential miscleavage. N-deamidation, M-oxidation, N-terminal acetylation and C-carbbamidomethylation were allowed as variable modifications. The solution of somathropin (1mg/mL) was infused into the source region of the MS for the intact analysis.

Results: The HDMS^{E} data were searched against a truncated database containg only somathropin sequence. The somathropin sequence coverage was 100%. The modification type, site and stoichiometry as well as retetion time of identified modified peptides are presented. The relative quantification of modified peptides calculated from HDMS^{E}

signal intensity shows that one methionine was change for cistyne. The spectrum of intact somathropin was showed to identify the protein and is possible to observe that impurities were detected.

Conclusion: The results presented here demonstrate with successfully the characterization of somathropin peptide maps with high sequence coverage and application of UPLC/HDMS^{E} . The Acquity UPLC and Synapt MS^{E} system meets requirements for robust and flexible methods that are needed to monitor safety and stability of bi biopharmaceutical protein. This method is potentially applicable for the quality control for somathropin during final product.