How to Identify Protein-Based Drugs in Equine Drug Testing?

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Protein-based drugs have been on the rise in recent years, as indicated by the increase in the number of such drugs approved by the United States Food and Drug Administration. Some of them may produce pharmacological effects attractive to athletes and racehorse trainers (or owners) as well as those who seek to have an advantage over others via drug doping. Among the protein-based drugs abused in horse racing are recombinant human erythropoietin (rhEPO) and its analogues, growth hormone, and cobra toxin. To deter abuse of protein-based drugs in horse racing, analytical methods for detection and identification of these drugs in equine plasma or urine samples are required. However, methodology for detection and identification of protein-based drugs is different from that of small-molecule drugs. In this presentation, approaches to identifying protein-based drugs relevant to equine drug testing will be presented. LC-MS/MS is the gold standard in drug testing. In view of method developments, various steps in an LC-MS/MS method for detection and identification of a protein-based drug in a biological matrix will be discussed. These steps include sample preparation, *in silico* and enzymatic digestions of a protein, liquid chromatography of resultant peptides, mass spectrometric analysis of the peptides, and assessment of the selectivity of a developed LC-MS/MS method for identification of a protein drug via bio-informatics tools such as BLAST protein database search and SEQUEST peptide product ion spectrum search. Examples of LC-MS/MS methods for identification of protein-based drugs will be provided.