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Identification of darbepoetin alfa in human urine by LC-MS/MS

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Abstract

For sports drug testing, the current analytical methods for recombinant human erythropoietin (rhEPO) are mainly gel electrophoretic methods, such as isoelectric focusing–polyacrylamide gel electrophoresis. Mass spectrometry is fundamentally necessary for the reliable identification of rhEPOs in doping control. In this study, a high-throughput mass spectrometric confirmation method for darbepoetin alfa (NESP) in human urine was established by a bottom-up approach. The novel method involves the immunopurification of human urine (10 mL), protease digestion with endoproteinase Glu-C (V8-protease) in an ammonium bicarbonate buffer (pH 7.8) and ultra-performance liquid chromatography using a charged surface hybrid C_{18} column coupled with electrospray-ionisation high-sensitivity tandem mass spectrometry for improved selectivity of the target molecules. The specific fragment peptide V_{11} (^{90}TLQLHVDKAVSGLRSLTTLLRALGAQKE^{117}) digested from NESP was monitored. The method was validated and the lower limit of detection of urinary NESP was 1.2 pg/mL. The limit of detection for identification was estimated to be 5 pg/mL. The developed method allows high-throughput confirmation analysis of NESP, namely 6 h for sample preparation and an analytical run time of only 10 min per sample. NESP could be identified in human urine collected after the intravenous administration of 15 µg NESP (n = 3). This mass spectrometric method is an innovative and powerful tool for detecting darbepoetin alfa in human urine for doping control testing.

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