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Mass spectrometric determination of gonadotrophin-releasing hormone (GnRH) in human urine for doping control purposes by means of LC-ESI-MS/MS

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Extended Abstract

The decapeptide gonadotrophin releasing hormone (GnRH) consists of ten amino acids (Pyr-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂, MW: 1181,58 Da) with a C-terminal amidation of glycine and cyclic-dehydration of glutamic acid at the N-terminus. It is endogenously produced in the hypothalamus and secreted into the microcirculation between the hypothalamus and pituitary gland in intervals of approx. 90 – 120 min. Here, the bioactive hormone is responsible for the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the systemic circulation. Because an intermittent application of exogenous GnRH in young males increases the testosterone plasma level by stimulation of the Leydig cells, the potential misuse of the administered substance offers a reasonable relevancy for doping controls and is prohibited in accordance with the list of prohibited substances of WADA. In contrast, with continuous hormone infusion or application of synthetic GnRH-analogues, inhibitory effects to the gonadotrophin release are dominant due to receptor down-regulation by persistent exposure.

The presented method provides a mass spectrometric approach to determine the non-degraded hormone in regular doping control samples by utilizing a sample preparation procedure with solid phase extraction, immunoaffinity purification with polyclonal antibodies coupled to magnetic beads and a subsequent separation by liquid chromatography with ESI-MS/MS detection. For liquid chromatography / mass spectrometry two alternative instruments were tested: the first consisted of an Agilent 1100 liquid chromatograph coupled to an Applied

Biosystems QTrap 4000 mass spectrometer, the second was a Waters Acquity nano-UPLC coupled to a Thermo LTQ Orbitrap high resolution / high accuracy mass spectrometer. Both instruments were found to be suitable with an enhanced performance for the nano-UPLC coupled to the LTQ Orbitrap device that is mainly substantiated by the increased sensitivity of the nano-scale ionisation interface.

In urine specimens provided from healthy volunteers, endogenous GnRH was not detected in accordance with the recent literature, but in post-administration samples (single application of Kryptocur 2 x 0.2 mg/spray, intranasal) urinary concentrations between 20 to 100 pg/mL of the intact peptide were determined. The method offered good specificity, linearity (5-300 pg/mL), limit of detection (LOD, approx. 5 pg/mL for the Waters Acquity nano-UPLC linked LTQ Orbitrap, 50 pg/mL for the Agilent 1100 linked API4000 QTrap), precision (inter/intra-day, < 20 %) and accuracy (105 %) using Des-pGlu₁-GnRH as internal standard to control each sample preparation step. In addition, the method transfer to other laboratories is not hindered due to commercial availability of all utilized chemicals and reagents.

Publication

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