PROJECT REVIEW

"Mass spectrometry characterization and identification of endogenous and synthetic insulins"

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The mass spectrometric characterization of endogenous as well as synthetic insulins provides the platform to reveal synthetic insulin misuse in sports as the modified analogues can be distinguished from endogenously produced insulin by molecular mass and diagnostic fragmentation pattern. The efficient isolation of various insulins from human plasma is accomplished by immunoaffinity chromatography followed by solid-phase extraction and allows an identification by liquid chromatography interfaced to mass spectrometry. This approach can be transferred to urine specimens, but improvements/modifications are required owing to the reduced concentration of intact insulins in urine samples compared to blood specimens. Hence, pre-concentration steps as well as parameter optimisation to assure utmost sensitivity of analytical instruments is necessary to enable the determination of synthetic insulins in urine samples. This procedure provides a complementary point and larger time frame to detect the abuse of synthetic insulins as growth promoting agents. Moreover, newly released synthetic insulins have to be considered and incorporated into the analytical assays for doping control purposes.

Results and Conclusions

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The rapid acting insulin analogues Lispro (Humalog™), Aspart (Novolog™) and Apidra (Glulisine™) are prohibited substances according to the Prohibited List of the WADA with exemption for athletes suffering from diabetes mellitus.

Although renal excretion of non-metabolised insulin was not demonstrated conclusively, method transfer for intact insulin determination from plasma to urine was successful. Commonly employed doping control specimens are urine samples and considering the short plasma half-life of the rapid acting analogues, urine analysis provides a sufficient tool to elucidate the abuse of these potentially performance-enhancing agents.

The developed method identifies and determines insulin and its analogues in urine by utilizing immuno-affinity-chromatography (IAC), solid-phase-extraction (SPE) and liquid-chromatography (LC) coupled to electrospray-ionisation-tandem-mass spectrometry (ESI-MS/MS).

Physiological amounts of excreted endogenous insulin for non-diabetics in urine were determined between 5 – 35 fmol/mL (30 – 200 pg/ml), hence these concentrations were assumed for insulin analogues as well. LC coupled with ESI-MS/MS ensure to distinguish the insulin analogues Aspart (5826 Da) and Apidra (5824 Da) from endogenous insulin (5808 Da) by product ion scan experiments of the five-fold protonated molecules. The identification of Lispro (5808 Da) and distinction from human insulin, which possess the same molecular mass and differ by the interchanging of Lysine and Proline in the amino acid sequence, was elucidated by small diagnostic fragment ions obtained from intact insulin as well as from the cleaved B-chain. The limit of detection of the developed method was calculated (S/N-ratio) as approximately 9 fmol/mL for each analogue. Recovery rates between 70 – 80 % were obtained and the precision at the LOD was found to be less than 20%. Specificity was proven for ten different urine samples fortified with IS while no interfering signals were detected, and in the concentration range of 9 - 35 fmol/mL linear approximation is permitted (Mandel). The "proof of principle" was shown by analysing doping control samples from athletes, suffering from diabetes mellitus, and declared administration of target insulin analogues.

Publication:

Thevis M, Thomas A, Delahaut P, Bosseloir A, Schanzer W. Doping control analysis of intact rapid-acting insulin analogues in human urine by liquid chromatography-tandem mass spectrometry. Anal Chem 2006;78:1897-903.