

PROJECT REVIEW

“Detection and Identification of Plasma Volume Expanders Based on Polysaccharide Structures in Human Urine Combined with the Analysis of the Diuretic Mannitol”

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A comprehensive method of sugar analysis which should include the plasma volume expanders HES (already published) and dextran and additionally the diuretic mannitol should extend and improve the possibilities of analysis in doping control urine samples. The scandals of the world championships in Lahti showed the necessity of the detection of this class of substances and, due to the close chemical similarity of some plasma volume expanders and mannitol, methods for the determination of abuse of these compounds should be established. Therefore, the development of sensitive and specific identification procedures are planned based on chemical analysis of excretion study urine specimen, reference compounds, consideration of earlier metabolism studies and routine analysis of doping control samples. Here, the suitability of gas chromatography and mass spectrometry was already proven with HES. The applicability of liquid chromatography coupled to mass spectrometry will also be tested with different ionization techniques. Concerning dextran the identification of the pattern of glucose and its polymers with 1,6-linkage should be elucidated and the origin of possibly occurring 1,6-linked oligo- or polysaccharides in human urine determined.

A validation of a method for identification and quantification of the 1,6-linked glucose as 1,5,6- triacetyl-2,3,4-trimethyl-glucitol will be performed. Normal values for this PMAA in urine of athletes in endurance sports will be established based on routine samples in the Cologne laboratory for doping analysis. Urine samples collected after application of mannitol and the different forms of dextran will be obtained from patients who are therapeutically treated with the medicaments.

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Results and conclusions

The intravenously administered plasma expander dextran and the diuretic agent mannitol are prohibited substances according to the “Prohibited list of substances” of the World-Anti-Doping-Agency. Plasma expanders are colloidal solutions that increase the blood volume by an influx of interstitial fluid. The plasma expander dextran is administered in cases of loss of blood, e.g. treatment of burns or hypovolaemic shock and for the stabilisation of the circulation of blood during narcosis. This “diluting effect” is of great interest in sports in order to control haematological parameters and masking of an EPO misuse. Mannitol is used as an osmotic diuretic by intravenous infusion to preserve renal function in acute renal failure and to reduce raised intracranial and intra-ocular pressure. In sports it may be administered to impair the excretion of prohibited substances.

Both dextran and mannitol are highly polar saccharide-based compounds. The related chemical properties of dextran, HES and the diuretic mannitol allow an implementation of the two compounds into the existing HES screening method. The screening method enables a semi-quantitative estimation of mannitol and glucose levels resulting from an entire hydrolysis of dextran.

For mannitol, a method based on GC-MS, which enables the detection and quantification of the analyte, was developed. The procedure allows distinguishing between the six stereoisomers of mannitol such as allitol, alritol, dulcitol, iditol, mannitol and sorbitol. Urinary mannitol concentrations could be determined following oral application of mannitol originating from commercially available sources (i.e. sweeteners). However, the method does not enable to distinguish between orally and intravenously administered mannitol.

A novel method, enabling the identification and quantification of the plasma volume expander dextran in human urine by LC-APCI-MS/MS was successfully developed. The concentration of dextran after intravenous application is 100-250 times higher than “normal” concentration levels of dextran (polymeric α -1,6-glucose) in human urine. Based on these results, a misuse of dextran in sports can be revealed by establishing a threshold level for dextran. Furthermore, an additional qualitative evidence for the presence of dextran can be accomplished by means of partially methylated alditol acetates (PMAAs) analysis, which provides precise information about the linkage positions of glucose monomers.

Publications and poster presentations

- 1) Thevis M, Guddat S and Schänzer W, Determination of HES, dextran and mannitol in human urine. In: Schänzer W, Geyer H, Gotzmann A, Mareck U, editors. Recent Advances in Doping Analysis (10) Köln: Sport und Buch Strauss, 2002:189-200.

- 2) Guddat S, Thevis M and Schänzer W, The diuretic mannitol – a problem in doping analysis. In: Schänzer W, Geyer H, Gotzmann A, Mareck U, editors. Recent Advances in Doping Analysis (11) Köln: Sport und Buch Strauss, 2003:323-328.
- 3) Guddat S, Thevis M and Schänzer W, Detection and quantification of the plasma volume expander dextran in human urine. Recent Advances in Doping Analysis (12) Köln: Sport und Buch Strauss, 2004.