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Detection and Quantification of the Plasma Volume Expander Dextran in Human Urine

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Extended abstract

Introduction

The intravenously administered plasma volume expander dextran is a prohibited substance according to the list of prohibited substances of the World Anti-Doping Agency (WADA)[1]. 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 [2]. Plasma expanders as colloidal solutions increase the blood volume by an influx of interstitial fluid. This "diluting effect" is of great interest in sports in order to control haematological parameters and masking of EPO misuse.

Dextran, an α-1,6-glucan, mainly consists of α-1,6-linked glucose molecules. A commonly employed method for the detection of urinary excreted dextran is the degradation and subsequent derivatisation of the polymer to partially methylated alditol acetates (PMAAs) [3-5]. This method will give precise information about the linkage positions of glucose monomers enabling the identification of dextran.

Due to the fact that α-1,6-linked glucose also occurs naturally, e.g. in starch, its presence in urine can not be excluded. With the analysis (PMAA) of 150 urine samples concerning the natural presence of 1,6-linked glucose, evidence for low basal levels of saccharides containing 1,6-linked glucose was obtained [6]. Thus, quantitation of the analyte was required in order to obtain evidence for an intravenous administration of dextran. An evaluation of the PMAA derivatisation procedure demonstrated difficulties in employing this method for quantitation purposes, hence we describe a novel assay based on enzymatic hydrolysis with dextranase [7] followed by derivatisation and liquid chromatography – atmospheric pressure chemical ionisation – tandem mass spectrometry (LC-APCI-MS/MS) for the identification and quantification of dextran in human urine.

Experimental

In a first step, dextran is decomposed by means of enzymatic hydrolysis with 1,6-α-D-glucan 6-glucano-hydrolase (dextranase) to the disaccharide isomaltose. In a following derivatisation step, the generated isomaltose is peracetylated. $^{13}C_6$-glucose was used as internal standard. All analyses were performed on a triple quadrupole mass spectrometer using the multiple-reaction-monitoring mode (MRM).

The method was used to determine the basal concentration of isomaltose, resulting from the enzymatic hydrolysis of polymeric 1,6-linked glucose in 238 routine doping control samples and the concentration of dextran, measured as isomaltose in 7 urine specimens obtained from patients treated with dextran.

In order to determine the concentration of isomaltose generated by enzymatic hydrolysis of dextran, urine samples were prepared twofold, with and without enzymatic hydrolysis. The concentration of isomaltose and maltose, occurring as "free" disaccharides in urine, obtained by the analysis of the sample prepared without enzymatic hydrolysis is subtracted from the concentration of isomaltose and maltose calculated in the sample prepared with enzymatic hydrolysis.

For quantitation purposes, the most abundant ion-transitions with *m/z* 696/169 and *m/z* 414/175 for peracetylated isomaltose and for peracetylated $^{13}C_6$-glucose respectively, were selected.

Results

Validation

Calibration curves for dextran were linear (ANOVA) and reproducible. The inter- and intra-assay CVs for dextran ranged from 4.9% to 7.3% at three concentration levels between 53 µg/mL and 1186 µg/mL. Recovery rate ranged from 97% to 112% (mean 106.9%). The absolute recovery for dextran was determined at 10.5% using peracetylated isomaltose as reference compound synthesized in our laboratory. The assay limit of detection was 3.9 µg/ml and the lower limit of quantification 12.5 µg/ml, respectively.

Basal concentration of isomaltose in human urine

For the determination of the basal concentration of isomaltose, resulting from the enzymatic hydrolysis of polymeric 1,6-linked glucose in human urine, 238 samples covering different sport sections were prepared with and without enzymatic hydrolysis. As shown in Figure 1, the concentrations of isomaltose were below or near the LOD in 209 (87.8%) and below LLOQ in 19 (8%) investigated samples. In 10 (4.2%) samples, the concentration of isomaltose ranged from 13.7 µg/mL up to 68.3 µg/mL, all of which were urine samples of cyclists and speed skaters.

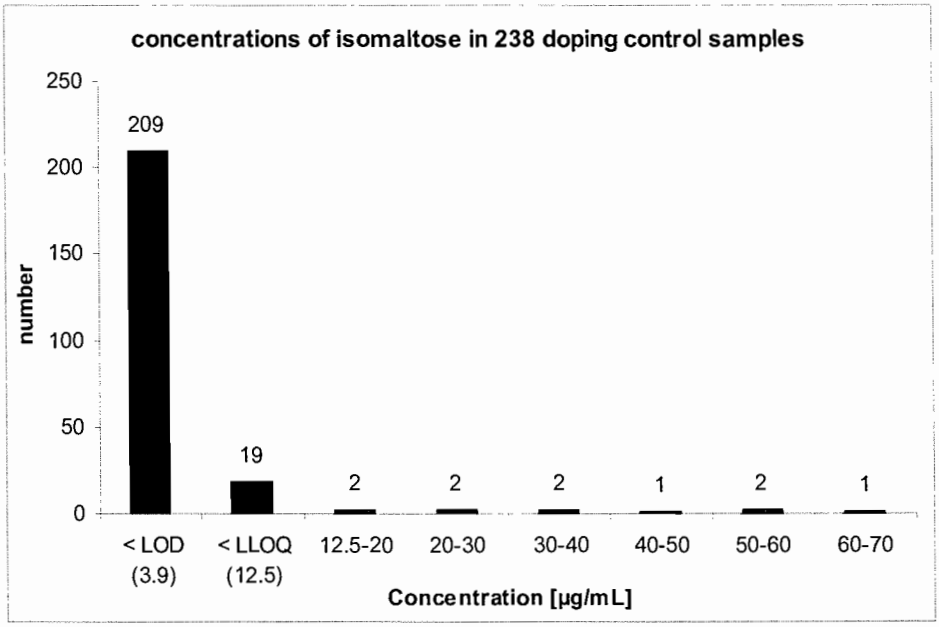

Figure 1: Basal concentration of isomaltose, resulting from the enzymatic hydrolysis of polymeric 1,6-linked glucose in 238 investigated doping control samples

The total concentration of isomaltose and maltose, composed of the "free" disaccharide fraction and the enzymatically generated isomaltose in the 238 analysed samples were not significantly increased in comparison to the basal concentrations of enzymatically derived isomaltose (Fig. 1).

Urinary concentration of dextran after intravenous application

For the determination of the concentration of dextran after intravenous application, 7 urine samples from patients treated with dextran were analysed. In the investigated samples, the concentration of dextran ranged from 7000 to 20000 µg/mL. The chromatogram shown in Figure 2 represents an analysed sample. The concentration of dextran was determined at 7.2

mg/mL, a value exceeding the highest concentrations of isomaltose found in 238 analysed doping control samples approximately by a factor of one hundred .

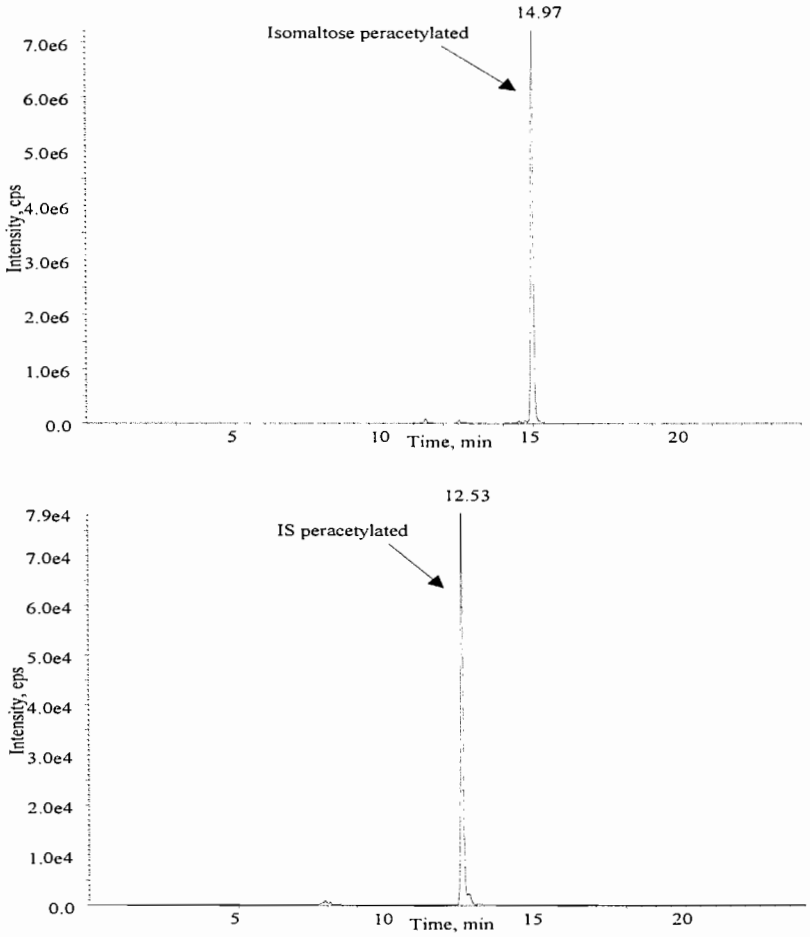

Figure 2: Extracted ion chromatograms of *m/z* 696/169 for Isomaltose and *m/z* 414/175 for IS representing an investigated urine sample of a patient treated with dextran

Discussion

The presented results indicate very poor urinary excretion of polymeric 1,6-linked glucose and "free" isomaltose or maltose. Even the highest concentrations of isomaltose (Fig.2) found in the analysed doping control samples were approximately 100 to 300 times lower as those determined in urine samples received from patients treated with dextran.

Owing to low amounts of polymeric 1,6-linked glucose naturally occurring in urine samples, a threshold level of 500 µg/mL is suggested for urinary dextran concentrations, enabling the identification of dextran administration. The presented results demonstrate the capability and reliability of the developed LC/APCI-MS/MS method for the identification and quantification of dextran in human urine. Distinct criteria for an implementation of the method into doping

controls are required in order to reveal a misuse of dextran in sports. An additional unambiguous qualitative evidence for the presence of dextran in suspicious urine specimens can be accomplished by means of PMAA-analysis.

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