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Screening and Confirmation Methods for the Detection of Plasma Volume Expanders in Human Urine

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Introduction

Since January 2000 plasma volume expanders belong to the list of prohibited substances of the International Olympic Committee[1]. This class of compounds includes polysaccharide-based medicals like hydroxyethyl starch (HES) and dextran, peptide-based medicals like crosslinked gelatines and also human albumine[2,3,4]. For HES two procedures were presented in 1999 and 2000 which enable the identification of the monosaccharides and their origin from a polymer[5,6]. Therefore, the procedures are not described again here.

Dextran is a polysaccharide of 1,6-linked α-D-glucose without any substitution and a branching degree of ca. 7% at the positions C-3 and C-4 (fig. 1). Its average molecular weight in remedies varies from 40.000 to 70.000 Dalton and it is usually administered as a 10% solution in 0.9% aqueous NaCl[2]. The metabolism of dextran differs from that of HES due to the lack of plasma enzymes able to degradate 1,6-linked glucose. Thus, the elimination is dependent on cell-bound enzymes cleaving the polymer into smaller units which can pass the renal barrier. Due to the comparable chemical properties of HES and dextran the analysis of the latter may be performed by the same procedures as used for HES. The other plasma volume expanders based on peptides mentioned above can not be included into the presented methods.

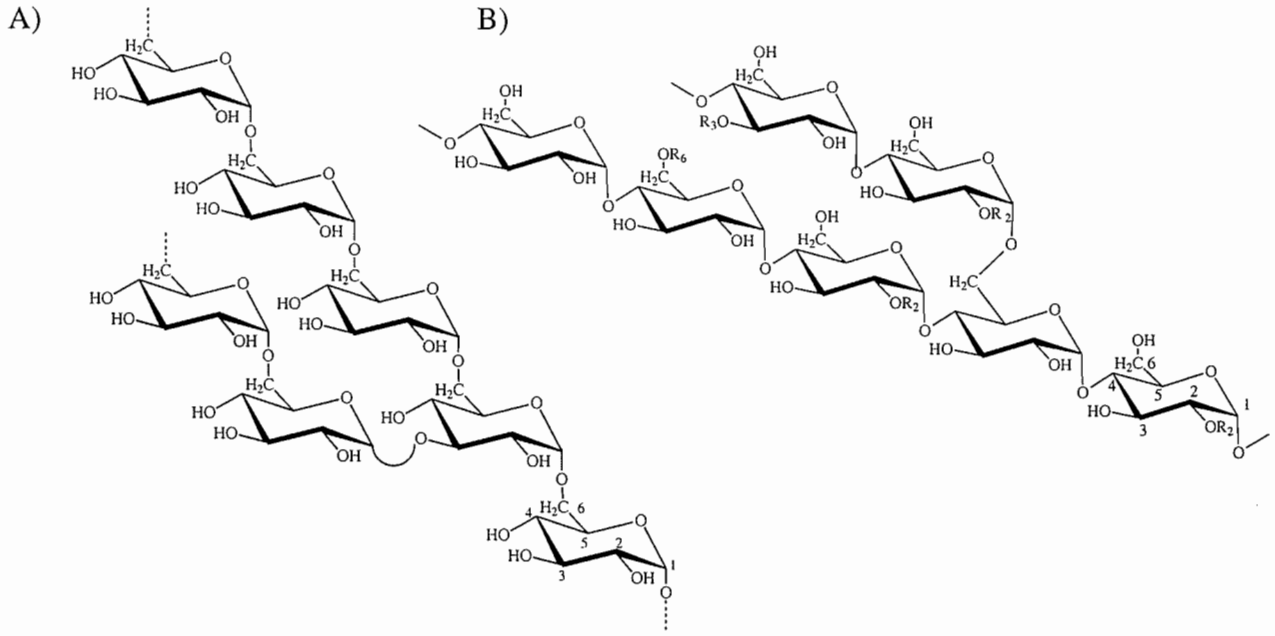

Figure 1: Structures of A) dextran and B) HES (R_2, R_3, R_6 = C_2H_5-OH)

Experimental

Chemicals

The reference of dextran was Rheomacrodex® 40 (10% in 0.9% NaCl) from Pharmalink (Sweden). Sodium borodeuteride was purchased from SIGMA. Methyliodide, dimethyl sulfoxide (dried), chloroform (for organic trace analysis), acetic anhydride, sodium borohydride (for synthesis), sodium hydroxide and glacial acetic acid were obtained from MERCK. Methanolic HCl was prepared by adding trimethylchlorosilane to methanol and shaking the solution for 5 minutes.

Sample preparation and GC/MS parameters

The preparation of blank urine specimen, dextran reference and excretion study urine samples of patients treated with dextran 40 was performed according to the methods described earlier[5-6]. The GC/MS parameters are also identical with those used in the previous studies with HES.

Results and discussion

The analysis of the dextran reference material and corresponding excretion study urine samples by means of acidic hydrolysis and derivatization to the per-TMS products of the screening procedure generated the expected intense signals for the α- and β-isomers of glucose. Those may serve as an indicator for a possible misuse of dextran in doping control urine specimen but do not provide sufficient information about the origin of the monosaccharides. The use of dextran can only be proven by the evidence of the original polymer and therefore the degradation and derivatization to the partially methylated alditol acetates (PMAAs) is reasonable. In figure 2 selected ion traces of the PMAA of 1,6-linked glucose in a chromatogram of an exctretion study urine sample are shown. The mass spectrum (figure 3) is significantly different from those generated by the PMAAs of e.g. 1,4-linked α-glucose or free urinary glucose and the compounds are also to distinguish by the retention times.

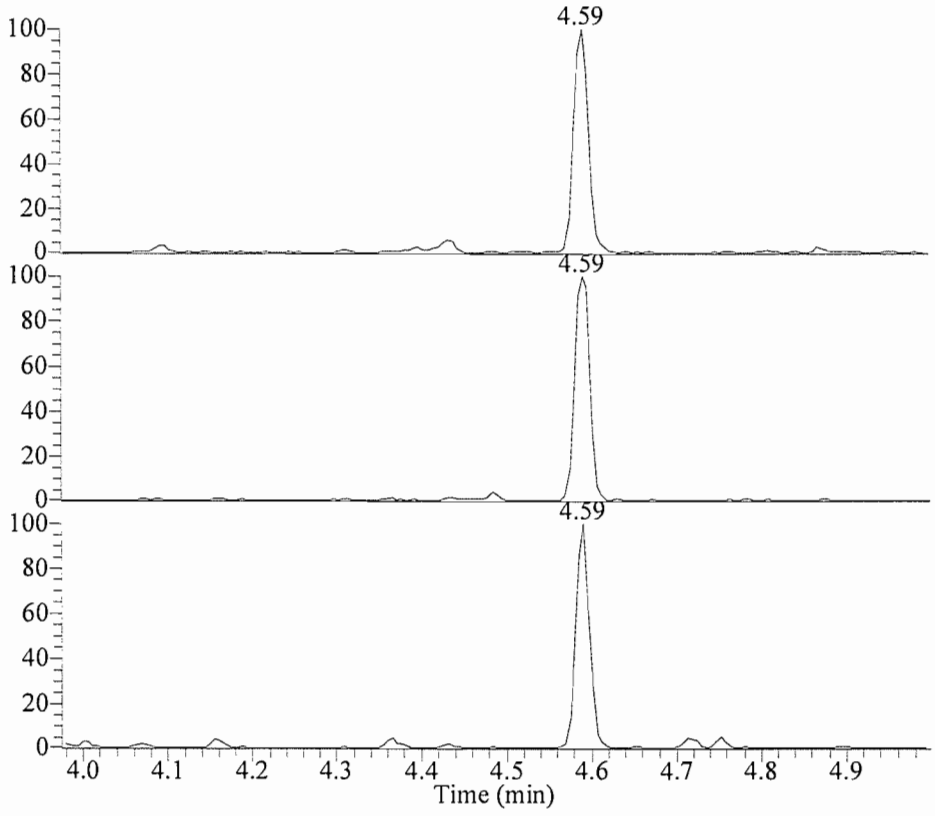

Figure 2: Selected ion traces of the PMAA of 1,6-linked α-D-glucose of an excretion study urine sample

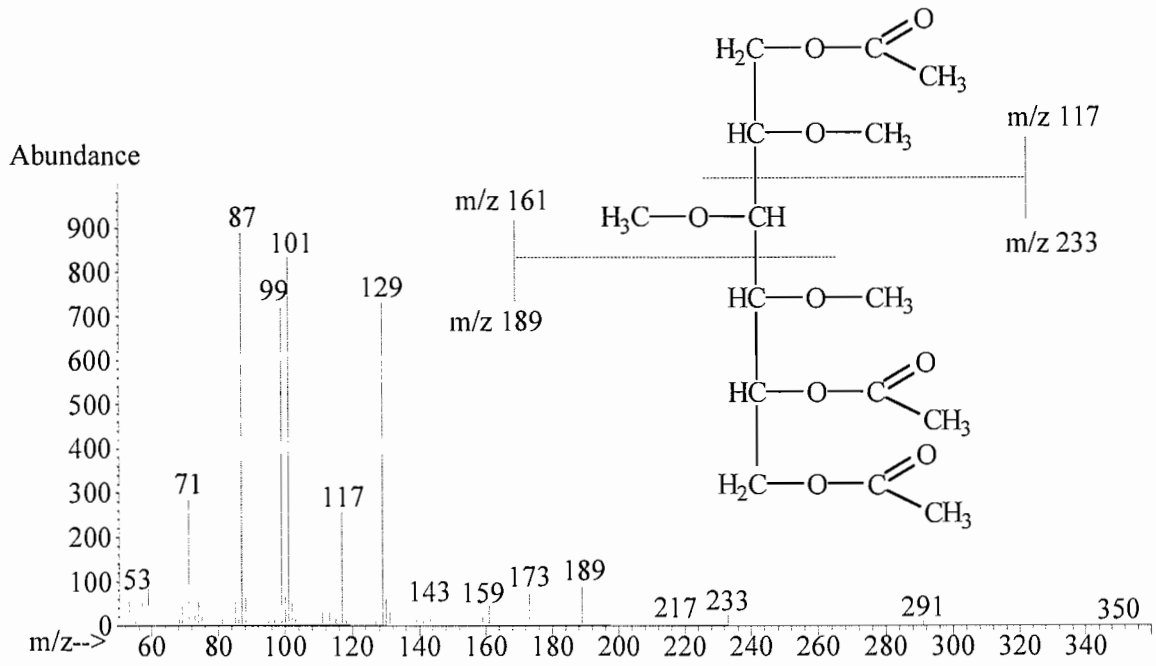

Figure 3: EI-mass spectrum of the PMAA of 1,6-linked α-D-glucose derived from dextran reference material.

The fragmentation scheme of the PMAA of 1,6-linked glucose appears in analogy to that of 1,4-linked glucose or its hydroxyethylated counterparts.[5] Here, the cleavage of the bond between C-2 and C-3, generating the fragments m/z 233 and 117, is not as intensive as with the PMAAs resulting from HES. This is owing to the fact that the fission between C-3 and C-4 now occurs competitively, generating the ions m/z 189 and 161. In case of 1,4-linked glucose the resulting PMAA bears an acetyl group at C-4, in contrast to the methyl group of the PMAA of 1,6-linked glucose. The product ions of m/z 189 are m/z 129 (after the loss of acetic acid, -60 amu) and m/z 87 by the subsequent elimination of ketene (-42 amu). The removal of acetic acid from m/z 161 generates the intense fragment m/z 101, and by the loss of methanol from m/z 161 the ion m/z 129 may also be produced in addition to its possible generation from m/z 189.

The analysis of 150 routine urine samples for dextran showed the presence of 1,6- and also 1,4-linked glucose residues in many cases. The concentration levels were far below those observed with the excretion study urine specimen but we can not exclude the possibility of naturally

elevated levels of 1,6-linked glucose in human urine. Therefore, a cut-off limit for the presence of 1,6-linked glucose would be very helpful.

Acknowledgements

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