Reprint from

# RECENT ADVANCES IN DOPING ANALYSIS (8)

W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck-Engelke
(Editors)

Sport und Buch Strauß, Köln, 2000

---

M.THEVIS, G.OPFERMANN, W.SCHÄNZER:
Rapid Screening Method to Detect Hydroxyethyl Starch (HES) in Human Urine
In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (8). Sport und Buch Strauß, Köln, (2000) 41-46

Mario Thevis, Georg Opfermann and Wilhelm Schänzer

# Rapid Screening Method to Detect Hydroxyethyl Starch (HES) in Human Urine

Institute of Biochemistry, German Sports University Cologne, Germany

## Introduction

The misuse of hydroxyethyl starch (HES, figure1) in high performance endurance sports was officially mentioned in 1998. In 1999 a procedure for its detection in human urine was presented providing unambiguous data about the administered plasma expanding agent but having a rather extensive sample preparation time [1-2]. Since january 2000 the IOC-doping list[3] prohibits the use of any plasma volume expander and a rapid screening method was developed, enabling a fast determination of the remedy in human urine[4].

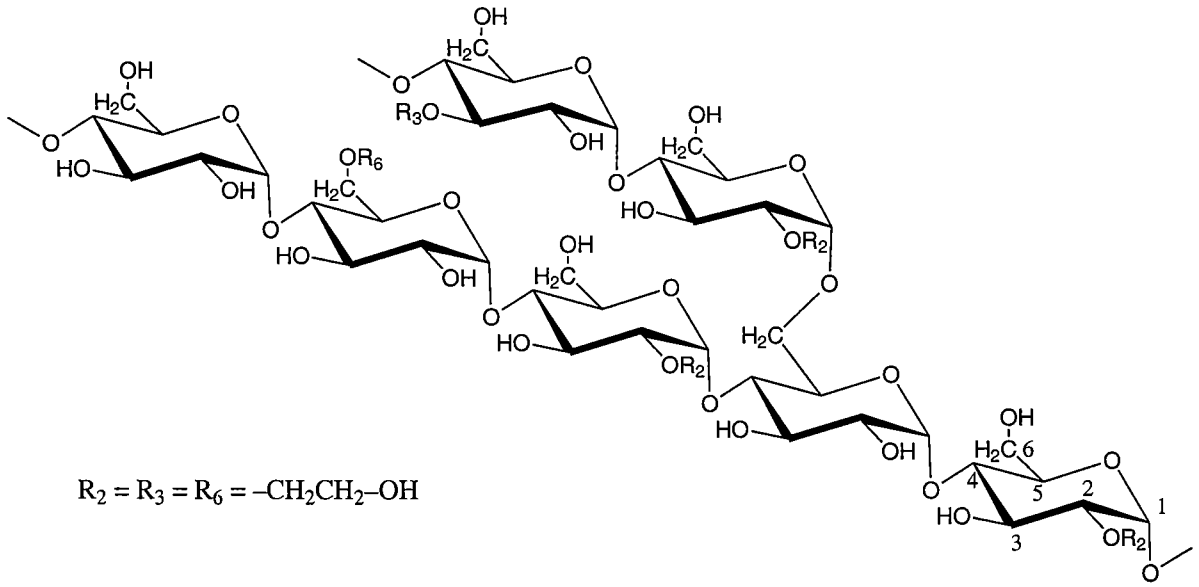
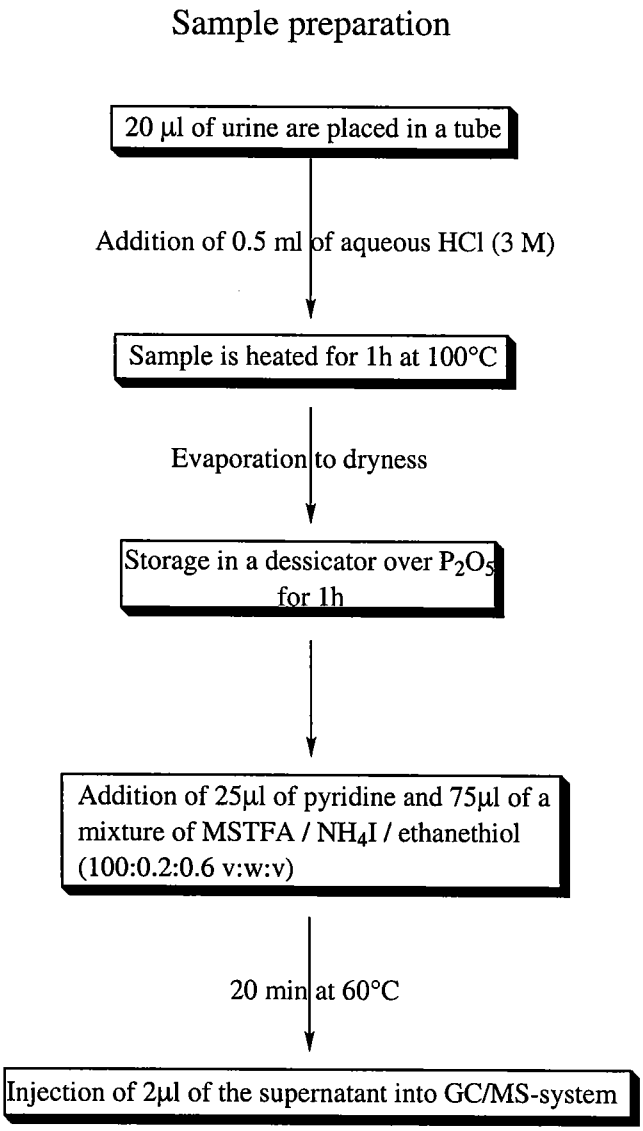



Figure 1: Hydroxyethyl starch

## Experimental

Chemicals:

N-Methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) was purchased from *Chem. Fabrik Karl Bucher* (Waldstetten, Germany) and distilled before use, ammonium iodide (purum, p.a.) was obtained from *Fluka* (Buchs, Switzerland), hydrochloric acid (32%) from *Merck* (Darmstadt Germany), ethanethiol (97%) from *Aldrich* (Deisendorf, Germany) and hydroxyethyl starch (HETASTARCH, 6% solution in 0.9% sodium chloride) from *Sigma* (Deisendorf, Germany).




Sample preparation:

20 µl of a urine sample is added to 0.5 ml of 3 M HCl in a test tube and heated for 1 hour at 100°C. After cooling to ambient temperature the mixture is evaporated to dryness and stored in a desiccator under reduced pressure over phosphorus pentoxide for 1 hour.

Then 25 µl pyridine and 75 µl of a mixture of MSTFA-$NH_4I$-ethanethiol (100:0.2:0.6, v:w:v) was added and the sample maintained at 60°C for 20 minutes. Finally 2 µl of the supernatand were injected into the GC/MS system.

GC/MS analysis:

The measurements were performed on an HP 6890/HP 5973 GC/MS system from Hewlett Packard (Waldbronn, Germany).

| | |
|---|---|
| Column: | HP 5MS capillary column, film thickness 0.25 µm, ID 0.25 mm, length 16 m |
| Carrier gas: | Helium 1.5 ml/min, split 1:10 |
| Injector temperature: | 300°C |
| Temp. program: | 0 min 140°C, +20°C/min, 2 min 320°C |
| Interface temp.: | 300°C |
| Ion source temp.: | 200°C |
| Ionisation: | EI (70 eV) |
| Mass range: | 50 - 550 amu |

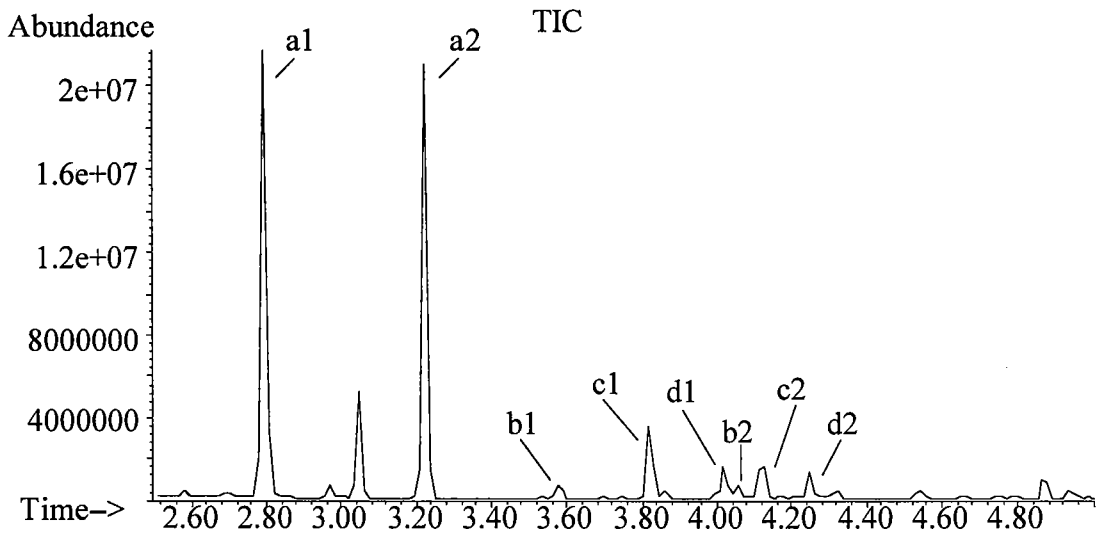
## Results

The acidic hydrolysis of the analyte (reference substance or urine aliquot) mainly yielded glucose, 2-, 3- and 6-hydroxyethylglucose with α- and β-isomers, due to the anomeric centre at C-1. Those were identified as per-TMS derivatives by gas chromatography / mass spectrometry as presented in the figures 2 and 3. The 2- and 3-hydroxyethylated glucose generate the typical fragment ions m/z 248, 261 and m/z 235, 248, respectively, which enable a rapid identification of the HES monosaccharides as shown in figure 2B. The mass spectra of the monomer units of HES are shown in figure 3. For more detailed information see reference 4.

## Acknowledgements

We thank the Bundesinstitut für Sportwissenschaft, Cologne, for the financial support and Dr. Willi Jansen (Dominikus Hospital, Düsseldorf) for organising excretion study urine samples.

A)




B)

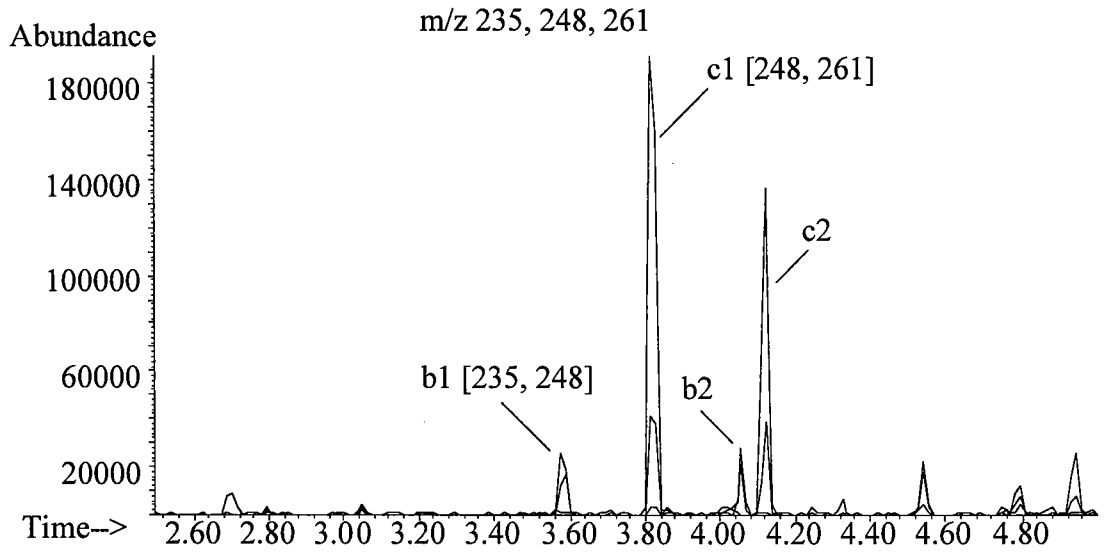



Figure 2: GC/MS Chromatogram of an HES reference standard: A) Total ion current (TIC), a1/a2 = α-/β-glucose per-TMS, b1/b2 = α-/β isomers of 3-hydroxyethyl-glucose per-TMS, c1/c2 = α-/β isomers of 2-hydroxyethyl-glucose per-TMS, d1/d2 = α-/β isomers of 6-hydroxyethyl-glucose per-TMS. B) Selected ion traces m/z 235, 248, 261.

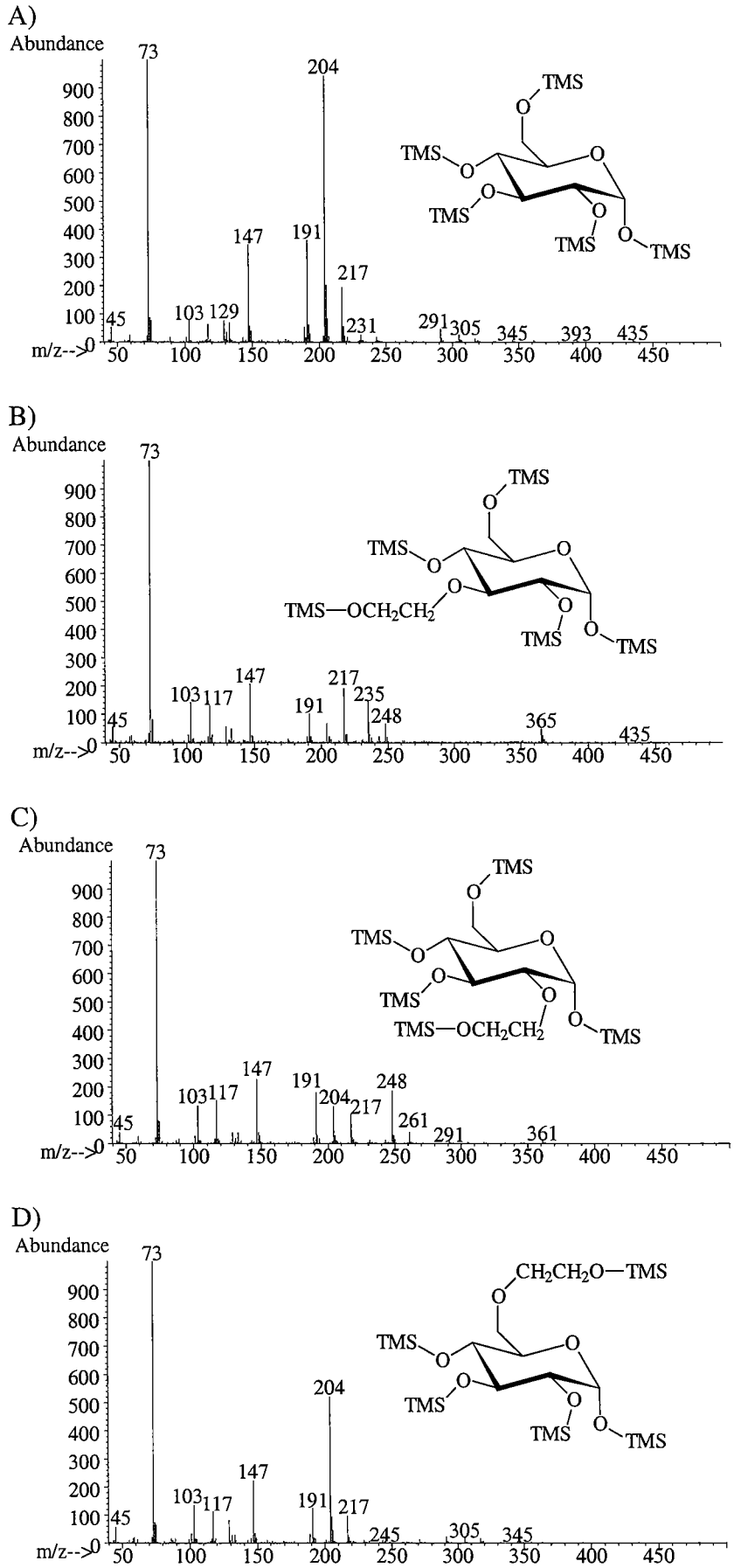



Figure 3:
EI mass spectra of A) α-glucose pentakis-TMS ($M_r$ = 540), B) 3-hydroxyethyl α-glucose pentakis-TMS ($M_r$ = 584), C) 2-hydroxyethyl α-glucose pentakis-TMS ($M_r$ = 584), D) 6-hydroxyethyl α-glucose pentakis-TMS ($M_r$ = 584).

## References

[1] Thevis M, Opfermann G, Schänzer W, *J. Mass Spectrom.* 2000, **35**: 77.

[2] Thevis M, Opfermann G, Schänzer W, in: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (Eds.) Proceedings of the 17th Cologne Workshop on Dope Analysis. Sport&Buch Strauß, Cologne 2000, pp 31-40.

[3] IOC List of Classes of Prohibited Substances and Methods of Doping, International Olympic Committee, Lausanne 2000.

[4] Thevis M, Opfermann G, Schänzer W, *J. Chromatog.* 2000, **744**: 345.