Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography B, 834 (2006) 217–220
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Detection of hydroxyethylstarch (HES) in human urine by liquid chromatography–mass spectrometry

K. Deventer*, P. Van Eenoo, F.T. Delbeke

Ghent University (UGent), Department of Clinical Chemistry, Microbiology and Immunology, Doping Control Laboratory (DoCoLab), Technologiepark 30b, B-9052 Zwijnaarde, Belgium

Received 27 October 2005; accepted 17 February 2006
Available online 20 March 2006

Abstract

The objective of this study was to establish the possibility of using liquid chromatography coupled to mass spectrometry for the detection of hydroxyethylstarch (a corn starch derived product) in urine as an alternative to the current time consuming GC–MS methods. Analyses were performed using an ion trap instrument after acidic hydrolysis. Ionization was carried out using atmospheric pressure chemical ionisation (APCI) operated in negative ionization mode and detection was performed using MS^2. The results indicate that the developed method can successfully be applied as a fast and reliable method for the detection and identification of hydroxyethylstarch.

Keywords: Doping; Liquid chromatography; Mass spectrometry; HES; Urine; Glucose

1. Introduction

Hydroxyethylstarch (HES) is a corn starch derived product. It is commercialised as a 6% solution of HES containing balanced electrolytes, lactate buffer and physiological levels of glucose.

HES is used as plasma volume expander in the acute treatment of shock or severe haemorrhage and as priming fluid for extracorporeal units [1]. Plasma volume expanders have the ability to retain water to the body by increasing the intravascular volume [2]. Hence this product gained interest amongst athletes taking part in endurance sports where dehydration is common. In sports where blood controls are performed (e.g.: cycling, skating) and where athletes are temporarily suspended if the hematocrite exceeds a certain level, HES can be used to increase the blood volume resulting in a decrease of the hematocrite value. The first positive cases for HES were reported during the Lahti Nordic World Championship (2001, Finland).

In doping control laboratories, HES is screened for by either mass spectrometric techniques including GC–MS [3,4] and MALDI-TOF [5] or colometric microtiter assays [6–8]. The latter technique is most often preferred for screening purposes because of the low cost and sample work up. However, to confirm the presence of HES in a urine sample, mass spectrometry must be applied. Due to the polar nature of HES identification by GC–MS requires a time consuming derivatization. During the last decade LC–MS has proven to be the ideal technique for the analysis of polar compounds and to be useful in the detection of monosaccharides [9–10]. As a result a sensitive method for the identification and quantification of dextran, another plasma volume expander, has been reported recently [11]. In this work the applicability of LC–MS for the detection and identification of HES in human urine was investigated.

2. Experimental

2.1. Chemicals and reagents

Hetastarch (6% Hydroxyethylstarch), glucose and glucose-$^{13}C_6$ were purchased from Sigma (Bornem, Belgium). To avoid degradation of HES and glucose standards, all stock

* Corresponding author. Tel.: +32 9 3313295; fax: +32 9 3313299.
E-mail address: koen.deventer@ugent.be (K. Deventer).
URL: http://www.docolab.ugent.be.

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.02.044

solutions and diluted solutions were prepared using a mixture of water/acetonitrile (80/20).

Analytical grade methanol, hydrochloric acid (HCl) and glacial acetic acid (HAc) were purchased from Merck (Darmstadt, Germany), HPLC grade acetonitrile (ACN) was from ACROS (Geel, Belgium).

2.2. Excretion samples

Excretion urine samples were obtained from different sources: two samples were obtained from the World Anti-Doping Agency (WADA), another sample was a kind gift from the German anti doping laboratory of Cologne.

2.3. Sample preparation

Sample preparation was based on a previously described method [4]. An internal standard solution (25 μl glucose-$^{13}C_6$, 1 mg/ml) and 100 μl HCl (3 M) were added to 100 μl urine followed by hydrolysis of the sample at 80 °C for 30 min. The sample was evaporated until dry under oxygen free nitrogen (OFN) at 80 °C. To prevent corrosion by HCl during evaporation, teflon coated needles were used. The remaining residue was dissolved in 200 μl mobile phase. Fifty microliter was injected in the chromatographic system.

2.4. Instrumentation

The HPLC system consisted of a P4000 quaternary pump equipped with a AS 3000 autosampler (all from Thermo Separation Products, Thermo, San Jose, CA, USA). The autosampler was equipped with a 100 μl sample-loop.

A Microsorb amine-type column 100 mm × 4.6 mm (3 μm) protected with a polar guard column 10 mm × 2 mm (both from Chrompack, Antwerp, Belgium), was used for chromatographic separation. The column was maintained at a temperature of 35 °C.

The mobile phase consisted of 1% acetic acid (in water) and ACN (20/80). Isocratic elution was performed at a flow rate of 0.5 ml/min (run time: 10 min).

Ionisation of the analytes was carried out on an LCQ-Deca mass spectrometer (Thermo, San Jose, CA, USA) using atmospheric pressure chemical ionisation (APCI). The capillary and vaporizer temperature were maintained at 150 and 350 °C, respectively. The discharge current was maintained at 5 μA and sheath gas and auxiliary gas were set at 80 and 10 (arbitrary units). Voltages of the capillary and lenses were optimized automatically. When MS^2 was applied the isolation width was set at 3.0, the activation q at 0.250 and the activation time at 30 ms. Ions observed during the MS^2-experiments are presented in Table 1.

Table 1
MS^2-settings and product ions for glucose-$^{13}C_6$ and ζ-hydroxyethylglucose in APCI negative ionisation mode

Compound	MW	PI	CE	Product ions
Glucose-$^{13}C_6$	186	245	20	185
ζ-Hydroxyethylglucose	224	283	25	223, 205, 161, 143, 113

PI, precursor ion; CE, collision energy; MW, molecular weight.

3. Results and discussion

LC–MS and GC–MS detection of HES is based on the detection of the monosaccharide units glucose and the structural isomers 2-, 3- and 6-hydroxyethylglucose (Fig. 1) after acidic hydrolysis of the polymerized HES. Due to their polar nature saccharides cannot be analysed using reversed phase liquid chromatography, hence an amine-type column was preferred [10].

In solution, glucose is in a permanent equilibrium between two forms, namely the α and β structure (anomers). Under the described conditions no chromatographic separation was observed between these two forms.

To determine mass spectrometrical parameters for HES, the monosaccharide glucose was infused. In ESI (positive and negative mode) no ions were detected for glucose. APCI operated in positive mode did not result in the detection of glucose neither. However, using full scan APCI in the negative mode $[M-H]^-$ and $[M+CH_3COO]^-$ ions could be detected. Analysis of a hydrolysed HES solution (100 μg/ml) resulted in the detection of 1 peak and analogous ions, i.e. taking into account the hydroxyethyl substituent. The identity of the structural isomers could not be determined due to the lack of individual reference compounds.

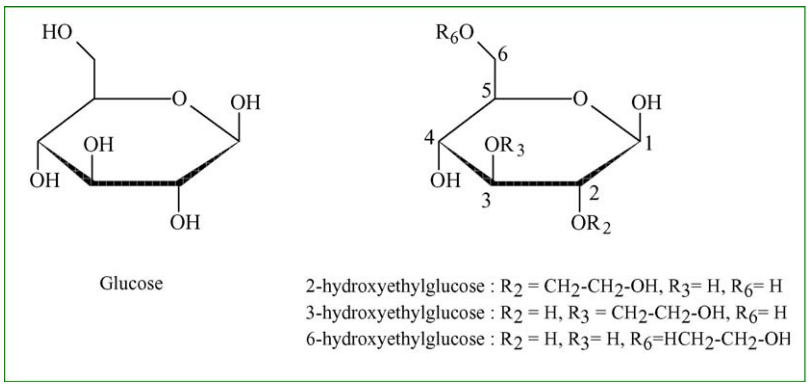

Fig. 1. Molecular structure for glucose and hydroxyethylglucose.

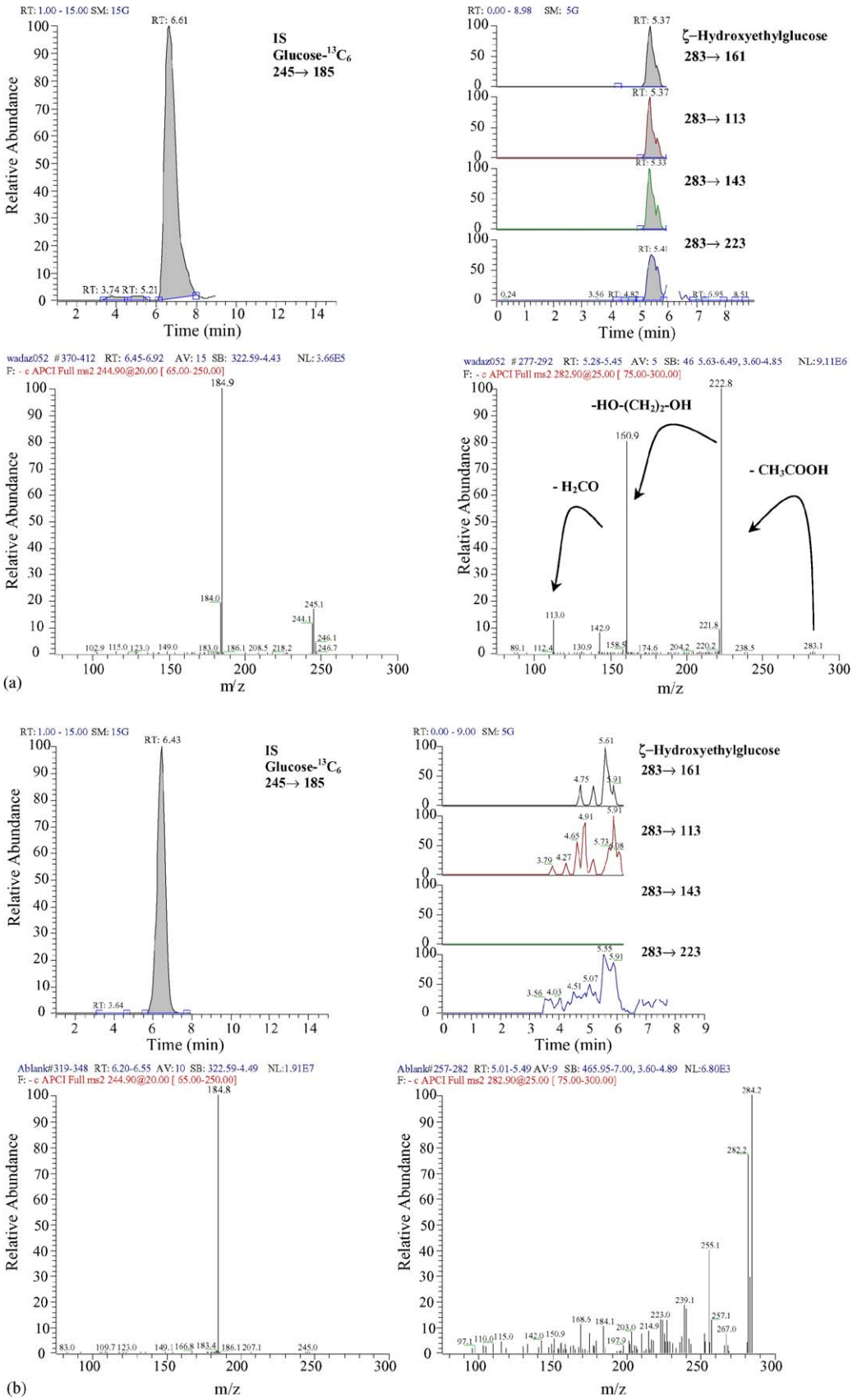

Fig. 2. Ion chromatograms and mass spectra for glucose-$^{13}C_6$ and ζ-hydroxyethylglucose obtained after analysis of an excretion urine sample (a) and a negative urine sample (b).

Because full scan LC–MS spectra contains few diagnostic ions, MS^2 was applied on the acetate adduct of hydroxyethylglucose. The most abundant ion observed in MS^2 was $[M-H]^-$ resulting from the loss of the acetate adduct (Fig. 2). Loss of the hydroxyethyl moiety, loss of formaldehyde and several smaller ions, resulting from the additional loss of water, could also be observed.

Chromatograms and product spectra for an excretion urine and a negative urine sample are presented in Fig. 2.

4. Conclusions

From this preliminary study it seems that LC–MS is a suitable technique for the detection and identification of HES and can be used as a simple alternative for the current GC–MS methods.

Acknowledgement

The authors are grateful to the Belgian National Lottery for the purchase of the LCQ-DECA® instrument. Grants by the Flemish Ministry of Health (KD and PVE) are greatly acknowledged.

References

[1] N.J. Wilkes, R.L. Woolf, M.C. Powanda, T.J. Gan, S.J. Machin, A. Webb, M. Mutch, E. Benett-Guerrero, M. Mythen, Anesth. Analg. 94 (2002) 538.
[2] C. Christidis, F. Mal, J. Ramos, A. Senejoux, P. Callard, R. Navarro, J.C. Trinchet, D. Larrey, M. Beaugrand, C.J. Guettier, J. Hepatol. 35 (2001) 726.
[3] M. Thevis, G. Opfermann, W.J. Schänzer, J. Mass. Spectrom. 35 (2000) 77.
[4] M. Thevis, G. Opfermann, W.J. Schänzer, J. Chromatogr. B 744 (2000) 345.
[5] R.G. Gallego, J. Segura, J. Rapid Commun. Mass Spectrom. 18 (1999) 1324.
[6] L. Avois, H.S. Lund, P. Hemmersbach, M. Saugy, in: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (Eds.), Recent Advances in Doping Analysis (12), Sport und Buch Strauβ, Köln, Germany, 2004, p. 371.
[7] R.G. Gallego, G. Such-Sanmartin, J. Segura, in: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (Eds.), Recent Advances in Doping Analysis (13), Sport und Buch Strauβ, Köln, Germany, in press.
[8] D. De Boer, personal communication, Laboratory for Doping analysis and Biochemistry, Lisbon, Portugal.
[9] A. Takatsu, S. Nishi, Biol. Mass Spectrom. 20 (1991) 415.
[10] T.S. McIntosh, H.M. Davis, D.E. Matthews, Anal. Biochem. 300 (2002) 163.
[11] S. Guddat, M. Thevis, W. Schänzer, Biomed. Chromatogr. 19 (2005) 743.