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Detection and confirmation of hydroxyethylstarch (HES) in human urine by liquid chromatography-mass spectrometry.

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Abstract

This paper describes a method for the determination and confirmation of hydroxyethyl starch by LC-MS APCI, using the instrument TSQ Vantage. The first step of analysis is the hydrolysis of the hydroxyethyl starch with hydrochloric acid. During the hydrolysis the hydroxyethyl starch is broken down to glucose and hydroxyethyl glucose that is the analyte. The adduct of the molecules of formic acid and analyte is used as a parent ion (m/z -269). The glucose $^{13}\text{C}_6$, in which all six carbon atoms are replaced by the isotope ^{13}C is used as the internal standard. Five typical product ions obtained by fragmentation of the mass of -269 were selected. MS-experiment was performed in SRM mode, mass-spectrometer parameters were optimized automatically to the maximum response signals of the analyte and the internal standard. It is experimentally proved that the molecule of hydroxyethyl glucose is detected only in the negative mode using APCI type of ionization. Amine-type column and mobile phase of the mixture of acetonitrile and 0.1 % formic acid were used at analysis procedure. The parameters of the gradient LC method have been optimized for maximum signal response of the analyte and lower influence of urinary matrix. This method requires less time for sample preparation and requires a small amount of chemicals, compared to the method described in the article of K. Deventer, P. Van Eenoo, F.T. Delbeke [1].

Introduction

Hydroxyethylstarch (HES) is one of the plasma volume expanders. HES is used in the medicine intravenously in cases of hypovolaemic shock that caused by dehydration, hemorrhage, burns and effects of drugs. It increases the blood volume, allowing red blood cells to continue to deliver oxygen to the body. Advantages of its use by athletes are different, e.g. the control of hematocrit, the retention of liquid in the body that prevents the decrease of the performance capability. HES is hydrolyzed to glucose and hydroxyethyl glucose (HEG). The aim of this work was to develop a method of detection and confirmation of HES by LC-MS.

Experimental

2.1. Chemicals and reagents

Refortan (6% hydroxyethylstarch) was purchased from Berlin-Chemie Menarini (Germany) and glucose $^{13}\text{C}_6$ from Campro scientific (Germany). All solutions were prepared using the HPLC-grade water and stored at 4°C.

2.2. Sample preparation

Hydrolysis was based on a previously described method [1,2]. An internal standard solution 25 μL glucose $^{13}\text{C}_6$ (100 $\mu\text{g}/\text{mL}$) and 150 μL HCl (6 M) were added to 100 μL of the urine followed by hydrolysis of the sample at 100°C for 60 minutes. After cooling to ambient temperature the sample was dried using a rotary evaporator under reduced pressure at a bath temperature of 60°C and the tube was stored in a desiccator under reduced pressure over phosphorus pentoxide for 30 minutes. The remaining residue was dissolved in 40 μL of the initial mobile phase. 35 μL were injected in the chromatographic system.

2.3. Validation

The MS-parameters optimization was made with hydrolyzed 100 µg/mL solution of HES because of lack reference standards of HEG. Three sets of 10 urine samples each containing HES (at 125, 250, 500 ng/mL) were prepared and analyzed for limit of detection purpose. 10 different blank urine samples were analyzed for the assessment of the effect of urine matrix.

2.4. Instruments and their parameters

MS: Thermo Scientific TSQ Vantage; Pump: Thermo Scientific Transcend 600 Pump; Column: Anion exchange from Agilent, Zorbax Sax 4.6 × 150 mm 5 µm; LC parameters: Flow rate: 0.6 mL/min, Mobile phase: (A) 0.1% HCOOH dissolved in water, (B) Acetonitrile; Time program: 0 min (10% A) → 11 min (100% A) → 12 min (100%, A) → 12.02 min (10% A) hold 3 min
MS parameters: Ion source: APCI; Nebulizer gas: 35 psi; Vaporizer temperature: 350 °C; Capillary temperature: 150 °C; Discharge current: 10 µA; Ion sweep gas pressure: 1.5; Collision gas pressure: 1 mtorr; S-lens RF amplitude: 55; Aux gas pressure: 5; Polarity: Negative; MS-experiment was performed in SRM mode. FIGURE2: shows the chromatogram of one of the ten negative urine samples.

Results and Discussion

The advantage of this method is, that it does not use chemicals to clean urine from protein fragments, and do not use filters MY-30 which is used in the article of K. Deventer, P. Van Eenoo, F.T. Delbeke [1]. Our method allows to reduce substantially time of sample preparation. Chromatography was performed on an amino type column. This kind of column is frequently used for the separation of saccharides [1]. It is experimentally proved that the molecule of HEG is detected only in the negative mode using APCI type of ionization. The glucose $^{13}\text{C}_6$ -185 m/z is used as the internal standard. We selected three typical product ions obtained by fragmentation of the -185 m/z (92, 123, 105 m/z with collision energy correspondingly 8, 8, 10 and Dwell 0.2). HEG and formic acid adduct formed the ion of -269 (224+46-1). The intensity of the adduct ion was higher than the ion intensity of the molecule of HEG (-223). Therefore, we used this adduct of the HEG for further analysis in the SRM mode. Five typical product ions (145, 205, 113, 45, 161 m/z with collision energy correspondingly 10, 7, 15, 67, 8 and Dwell 0.2) obtained by fragmentation of the mass of -269 were selected. FIGURE1 shows the chromatogram of one of the ten urine samples containing HES at the concentration of 0.25 µg/mL. The peak of HEG was observed in all spiked urine samples at concentrations of HES starting from 125 ng/mL. So the limit of detection of this method is not above than 125 ng/mL. FIGURE2 shows the chromatogram of one of the ten negative urine samples. The peak with retention time of HEG was not observed in all ten blank samples. So the specificity of this method is good.

Conclusions

Analysis of the hydrolyzed HES solution resulted in the detection of 1 peak, though the hydrolysis of HES gives at least three isomers (2-OH, 3-OH, 6-OH) of HEG [1]. This type of column does not allow split three isomers. The identity of the structural isomers could not be determined due to the lack of individual reference compounds. But, because of lack such peak in blank urines, the method suitable for detection of HES. This method can be used as the method for confirmation of the presence of HES in urine samples by using anion exchange column and MS/MS experiment with APCI ion source in negative mode, and the method eliminates the step derivatization [3].

References

1. K. Deventer, P. Van Eenoo, F.T. Delbeke, Recent Advances in Doping Analysis (14). Sport und Buch Strauß, Köln, Germany (2006) 71-78.
2. J.Son, D.-H. Kim, M.-J. Kang, M. H. Choi, Recent Advances in Doping Analysis (14). Sport und Buch Strauß, Köln, Germany (2006) 423-426.
3. S. Guddat, M. Thevis, A. Thomas, W. Schänzer, Recent Advances in Doping Analysis (16). Sport und Buch Strauß, Köln, Germany (2008) 71-72.

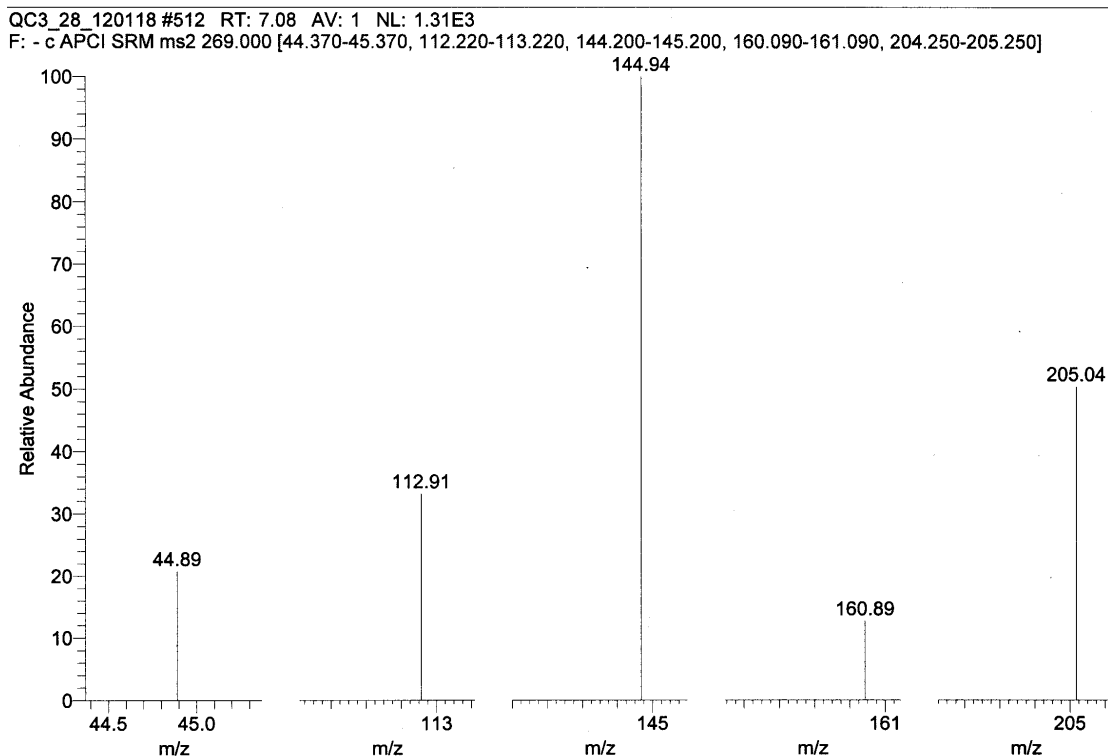
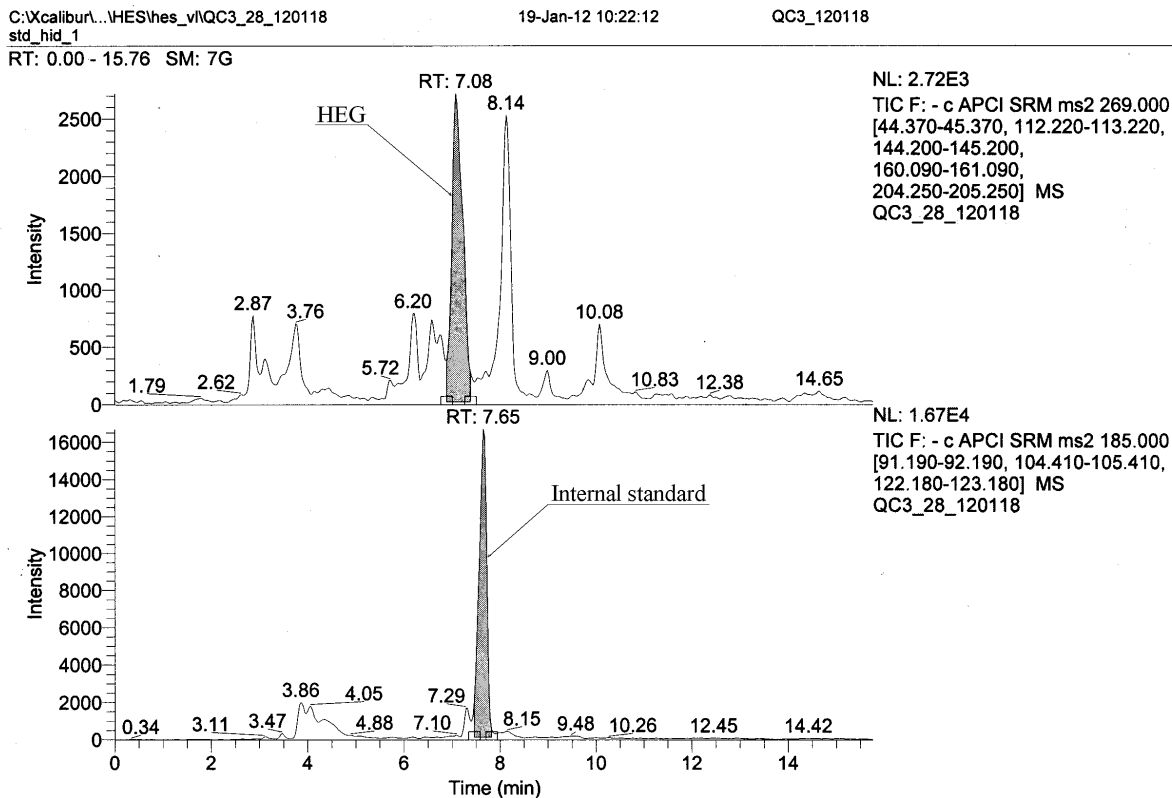
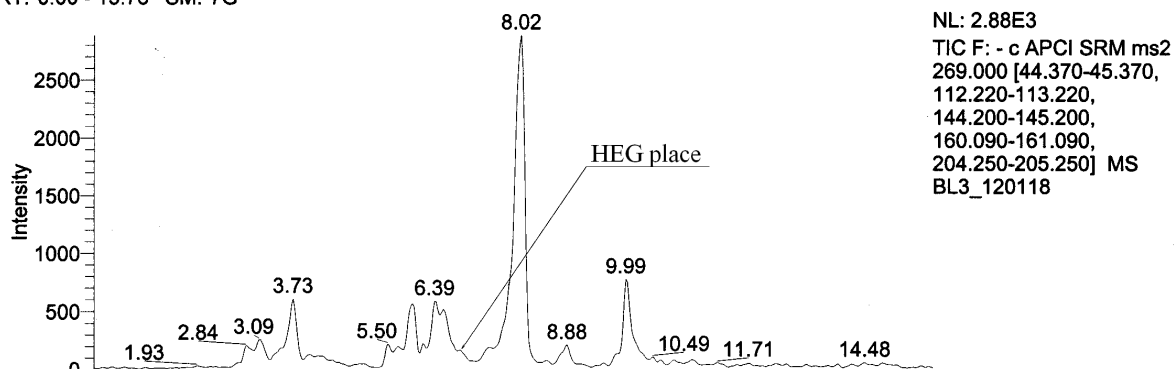


FIGURE1: shows the chromatogram of one of the ten urine samples containing HES at the concentration of 0.25 µg/mL.

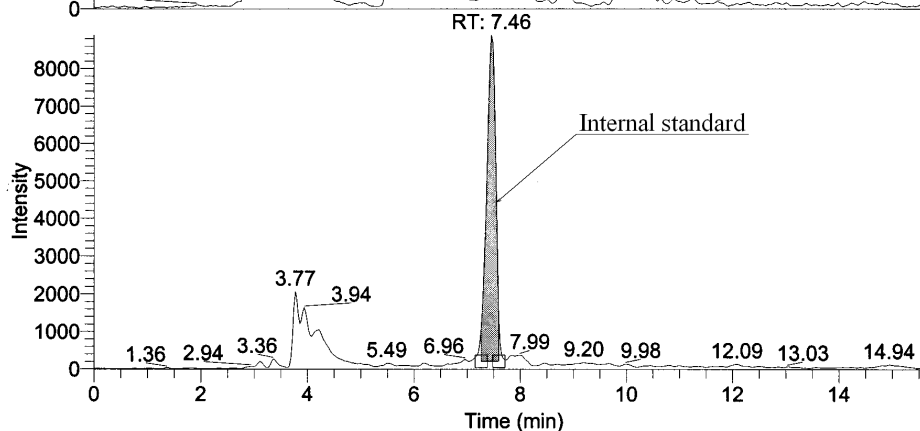
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BL3_120118



NL: 2.88E3
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269.000 [44.370-45.370,
112.220-113.220,
144.200-145.200,
160.090-161.090,
204.250-205.250] MS
BL3_120118



NL: 8.89E3
TIC F: - c APCI SRM ms2
185.000 [91.190-92.190,
104.410-105.410,
122.180-123.180] MS
BL3_120118

BL3_120118 #498 RT: 6.89 AV: 1 NL: 7.59E1
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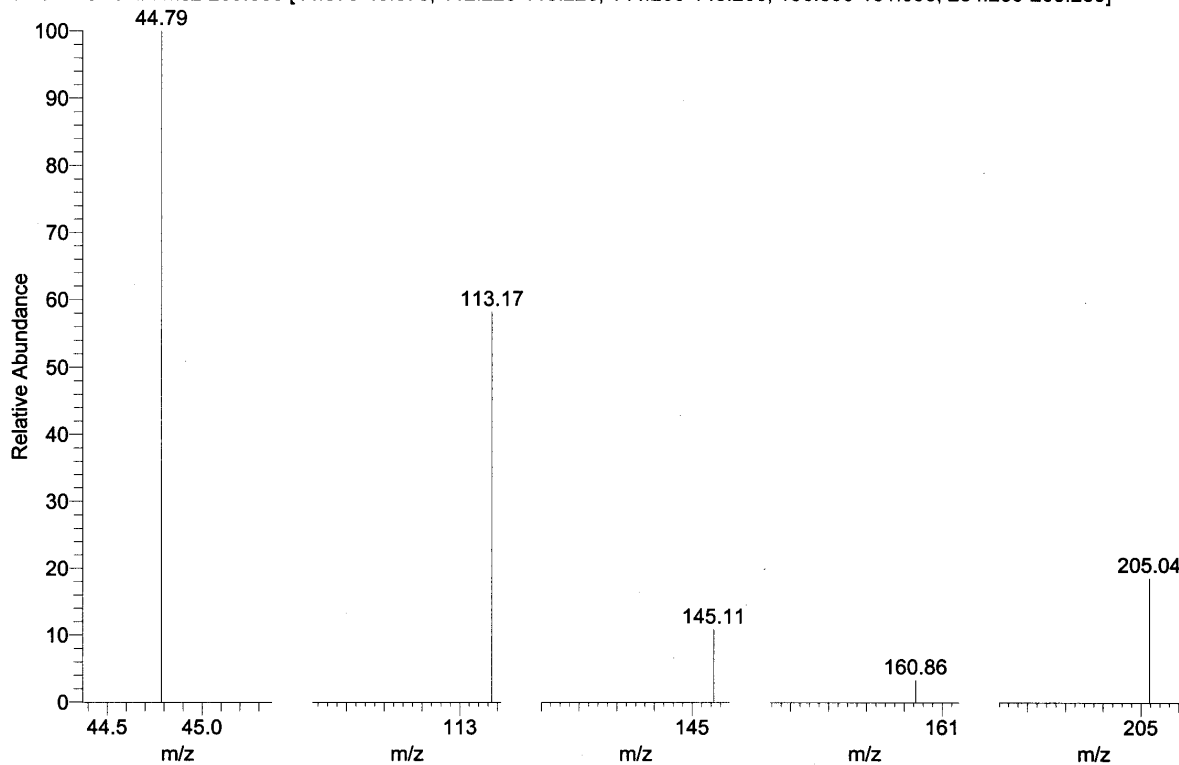


FIGURE2: shows the chromatogram of one of the ten negative urine samples.