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Detection of Glycerol in Human Urine Using the Screening Procedure for Hydroxyethyl Starch and Dextran

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Abstract

Since 2010 glycerol has been included in the WADA list of prohibited substances in the class S5 (Diuretics and Other Masking Agents). Starting in 2013 it has been designated as a Threshold Substance, with a threshold value of 1 mg/mL. The procedure for the detection of glycerol is very similar to that for the plasma expanders, hydroxyethyl starch (HES) and dextran. Namely it involves the aliquoting of a small volume (20 µL) of urine sample, acid digestion and drying. All three compounds are then derivatized with MSTFA/NH_4I/2-mercaptoethanol (1000:2:6, v/w/v) and separated by GC-MS.
The inclusion of glycerol in the screening procedure for HES and dextran requires a modification of the GC temperature program. The effect of the acid digestion step was checked to show that it had no effect on the stability of glycerol. Combining all three compounds into one procedure leads to a saving of analysis time and equipment.
This method has been applied to urine samples receieved by the Centre during the period April to June 2013.

Introduction

Glycerol forms the backbone of triglycerides and phospholipids. In humans, normal plasma levels are *ca*. 0.05 mM. The administration of glycerol has been prohibited by WADA since 2010 [1]. It is classified as a plasma volume expander (S5 Diuretics and Other Masking Agents). In 2013, it has been designated as a Threshold Substance, with a threshold value of 1 mg/mL [2]. The aim of this work was to include glycerol into the screening procedure for hydroxyethyl starch (HES) and dextran. The developed method has been applied to screening of samples from Thai athletes during the period April to June 2013.

Experimental

1) Sample preparation

20 µL of urine sample and 25 µL of $^{13}C_6$ -glucose (1 ppm, IS) was hydrolyzed with 3M HCl (100 µL) at 100 °C for 1 hr and then dried under N_2 stream at 65 °C. The residue was then placed in a vacuum oven at 40 °C and then derivatized with 75 µL of MSTFA/NH_4I/2-mercaptoethanol (1000:2:6, v/w/v) and 25 µL of pyridine at 60 °C for 20 min. The sample was then transferred to GC vial and injected into the GC/MS system [3].

2) Instrument conditions

Instrument: Agilent 6890 (GC) and 5973N (MSD)
Column: ZB-1 (20 m x 0.25 mm x 0.10 µm)
Injection parameters: Injection mode: Split (10:1), Inlet temperature: 300 °C
Temperature program: Initial temperature: 95 °C, ramp 10 °C /min to 140 °C, ramp 20 °C /min to 320 °C, hold 2 min
MS parameters: Ionization mode: EI at 70 eV, Interface temperature: 320 °C , Source temperature: 230 °C, Acquisition mode: SIM, Glycerol (m/z 205, 218, 293); Hydrolyzed HES, dextran (m/z 191, 192 (IS), 204, 206 (IS), 217, 220 (IS), 235, 248, 261)

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Results and Discussion

It was found that the screening procedure for HES and dextran, with a modified temperature program, could detect all 3 compounds, as shown in the TIC and mass spectra (Figure 1A, 1B, 1C). The retention time and characteristic ions of glycerol, hydrolyzed HES and dextran for the positive control urine (UPC) are listed in Table 1.
This procedure was applied to the analysis of 296 urine samples from Thai athletes during April to June 2013. All the data are shown in Table 2 and Figure 2. There were 157 samples (54%) with the concentrations of glycerol lower than 0.25 mg/mL. The median concentration of glycerol was 0.22 mg/mL, with levels ranging from undetectable amounts to the maximum value of 0.96 mg/mL.

Compound	RT (min)	Characteristic ions (m/z)
Glycerol	2.978	218, 205, 293
α-Glucose $^{13}C_6$ (IS)	7.816	206, 192, 220
β-Glucose $^{13}C_6$ (IS)	8.316	206, 192, 220
α-Glucose	7.816	204, 191, 217
β-Glucose	8.316	204, 191, 217
3α-OH-ethylated glucose	8.649	235, 217, 248
3β-OH-ethylated glucose	9.159	235, 217, 248
2α-OH-ethylated glucose	8.904	248, 217, 261
2β-OH-ethylated glucose	9.218	248, 217, 261
6α-OH-ethylated glucose	9.071	204, 191, 217
6β-OH-ethylated glucose	9.325	204, 191, 217

Table 1: The GC retention times (RT) and characteristic ions of glycerol, hydrolyzed HES and dextran

Concentration range (mg/mL)	Number of samples
ND-0.25	157
0.26-0.50	71
0.51-0.75	52
0.76-1.00	16
> 1.3	0
Total N	**296**

ND : not detected

Table 2: Concentration range of glycerol in urine samples

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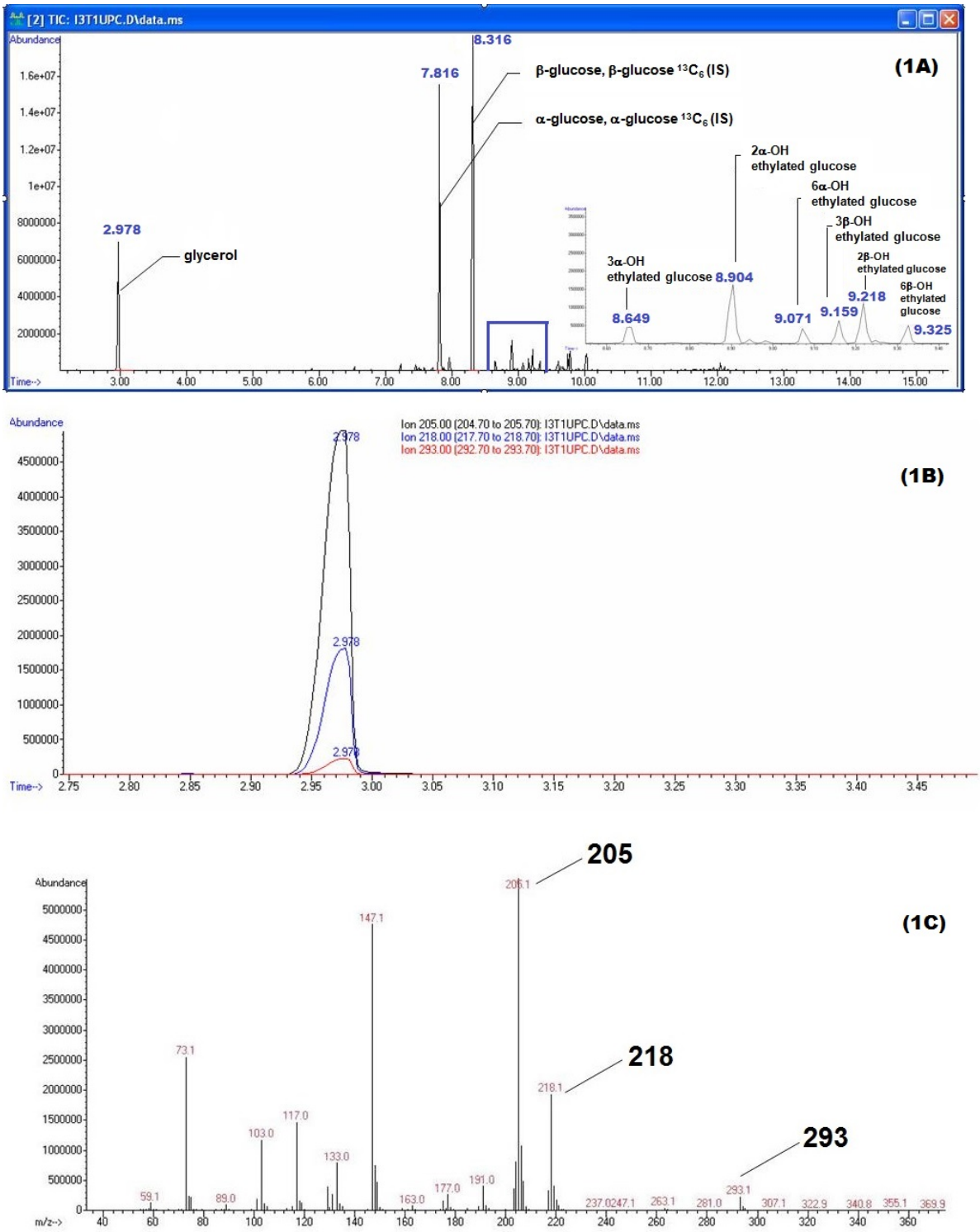

Figure 1: **(1A)** TIC of the spiked control urine at 1 mg/mL of glycerol and 2.4 mg/mL of HES and dextran ; **(1B)** Extract ion chromatogram (SIM mode) for glycerol-tris-TMS ; **(1C)** Full SCAN mass spectrum of glycerol-tris-TMS

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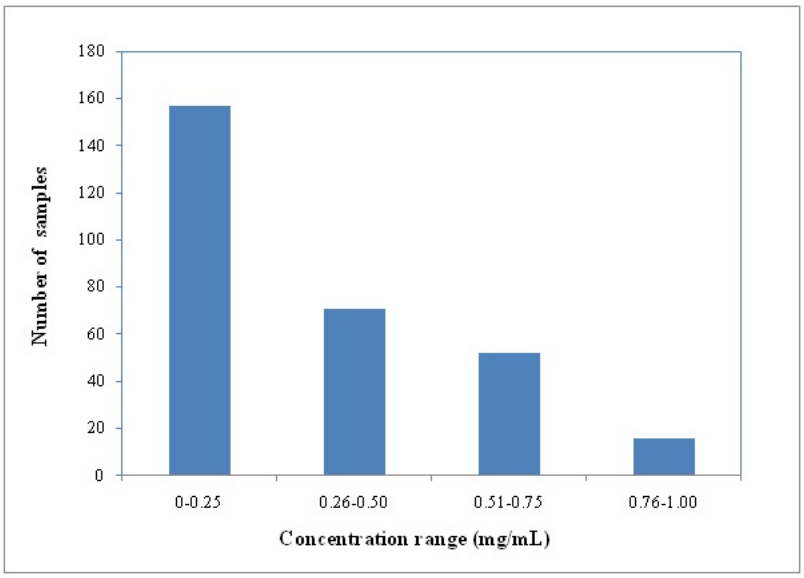

Figure 2: Histogram for the concentration range of glycerol in 296 urine samples

Conclusions

A modification in the GC temperature program in the screening procedure for HES and dextran enabled glycerol to be detected. Since there is an acid digestion step for HES and dextran, it was found that there was no effect on the stability of glycerol. The combination of analysis of three compounds in one procedure leads to saving of time and equipment.

References

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