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The Diuretic Mannitol – A Problem in Doping Analysis
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The diuretic mannitol-a problem in doping analysis

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1. Introduction

The intravenously administered diuretic agent mannitol (Fig. 1) belongs to the list of prohibited substances of the International Olympic Committee (IOC), and from January 2004 the list of the World-Anti-Doping Agency (WADA) will also include it.

The sugar alcohol infusion as an osmotic diuretic by intravenous infusion in order to preserve renal function in acute renal failure and to reduce raised intracranial and intra-ocular pressure[5].

Within the framework of a project supported by WADA, mannitol is identified and quantitated in human urine.

Several methods for identification and quantification of mannitol in urine are described in the literature using e.g. high-performance anion-exchange chromatography with electrochemical detection [3,4] or gas-chromatographic mass spectrometric techniques. In the present study, a GC-MS method was developed based on acetylation of the analytes (Thevis *et al.*, 2000), which allows the separation of mannitol from its stereoisomers, as well as identification and quantitation of mannitol in human urine. Mannitol excretion study urine samples were obtained from healthy volunteers and analysed in order to estimate the influence of orally administered mannitol on its urinary concentration.

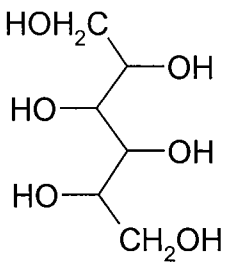

Figure 1: Structure formula of mannitol

2. Experimental

Separation and identification

For the quantitation of mannitol it is necessary to assure separation of mannitol from its stereoisomers, sorbitol, iditol, altritol, allitol and dulcitol. A suitable way consists of its derivatisation to hexa-acetylated mannitol and subsequent measurement by GC-MS.
Mannitol was identified by measuring all single hexa-acetylated alditols, comparison of their retention times to $^{13}C_1$-mannitol, and by increasing each signal by addition of its corresponding hexa-acetylated alditol.

Sample Preparation

A volume of 10 µl of an aqueous solution of mannitol, sorbitol, iditol, altritol, allitol and dulcitol (c = 1mg/ml) is placed in a test tube, 20 µl of a blank urine and 20 µl of an aqueous $^{13}C_1$-mannitol solution (c = 50mg/ml) are added. The solution is dried in a desiccator over phosphorus pentoxide under reduced pressure for at least 3 hours. For acetylation, the residue is solved in 350 µl of acetic anhydride - acetonitrile – pyridine (3 : 3 : 1, v / v / v) and heated for 2 h at 80 °C. A volume of 2 ml of distilled water and 2 ml of chloroform are added, the sample is vortex mixed and the aqueous layer is removed. The remaining chloroform layer is evaporated to dryness under reduced pressure at 50 °C, and the residue is dissolved in 100 µl of 2-propanol. 2 µl of this solution are injected into the GC-MS system.

GC-MS analysis

All analyses were performed on a Hewlett Packard 6890/5973 GC-MS system. Conditions were as follows: column: HP 5-MS capillary column, film thickness 0.25µm, 16m x 0.25 mm I.D.; carrier gas: helium, 1.5 ml/min, split 1:10; injector temperature: 300 °C; The temperature program starts at 140 °C, increasing with 10 °C/min to 220 °C , followed by an increase with 40 °C/min to 320 °C. The interface temperature was 280 °C; ion source temperature: 230 °C; Ionisation was performed by electron impact ionisation (EI) (70eV), and a mass range of 50-600 u was recorded.

3. Results

All 6 alditols were adequately separated as demonstrated in Fig. 2 and characteristic fragment ions were generated upon EI ionisation as shown in Fig. 3.

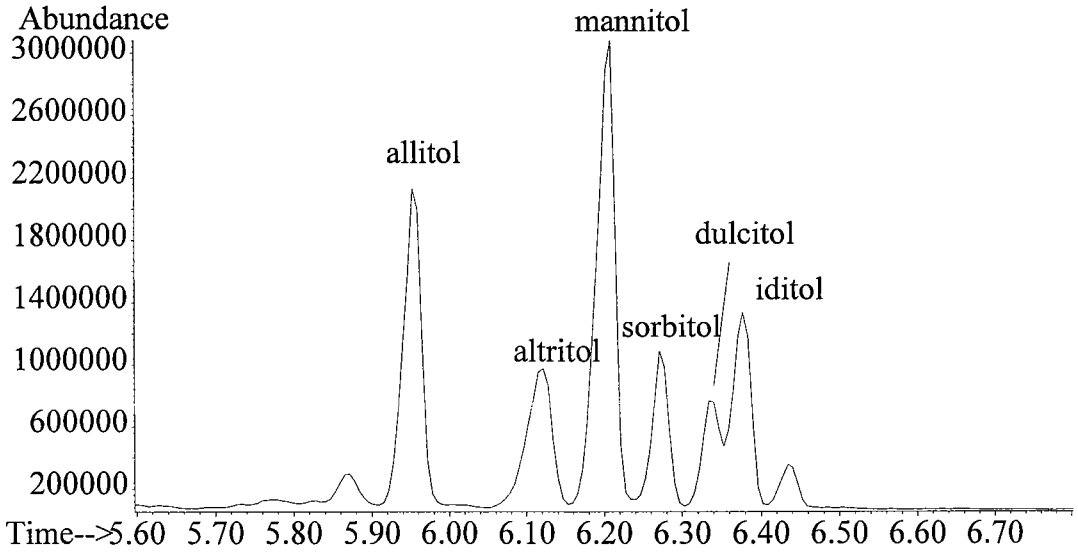

Figure 2: GC-MS chromatogram of the per-acetylated alditols

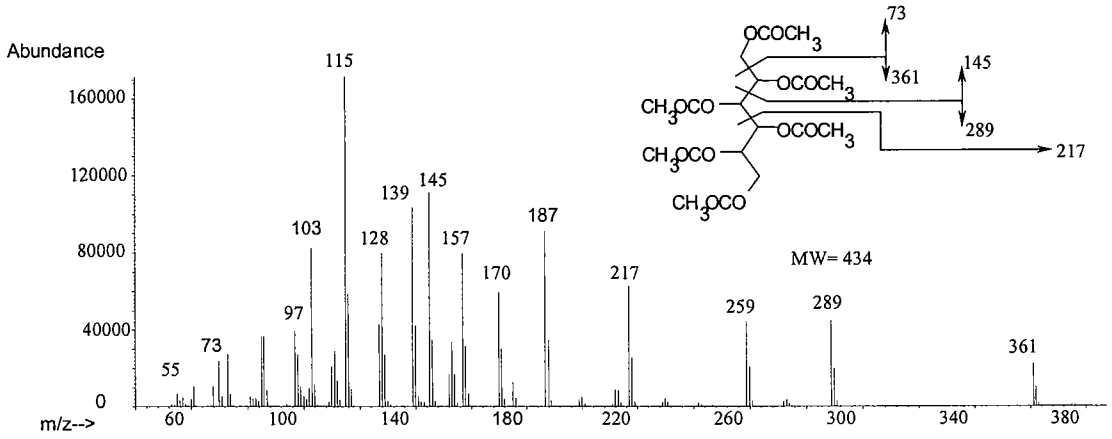

Figure 3: EI mass spectrum of hexa-acetylated mannitol

Quantitation

For the quantitation of mannitol, a calibration curve was prepared from 30 ng/µl to 10 µg/µl. This calibration curve enables the quantitation of mannitol by comparison of peak areas of the ion trace at *m/z* 289 of mannitol and the internal standard (allitol).

Regression line: y = 0.0577x - 9.6496, R^2 = 0,9987

The calibration curve is linear within the selected range. Linearity was tested according to DIN 38402, Teil 51 (German industry norm 38402, chapter 51)

Excretion study

Mannitol excretion study urine samples were obtained from 3 male and 3 female healthy volunteers. After oral application of 10 g of mannitol, aliquots were collected every 2 hours over a period of 8 hours and a last one after 24 hours. The measured concentrations are shown in Fig. 4, and the total amount of mannitol excreted at respective collection times are demonstrated in Fig. 5.

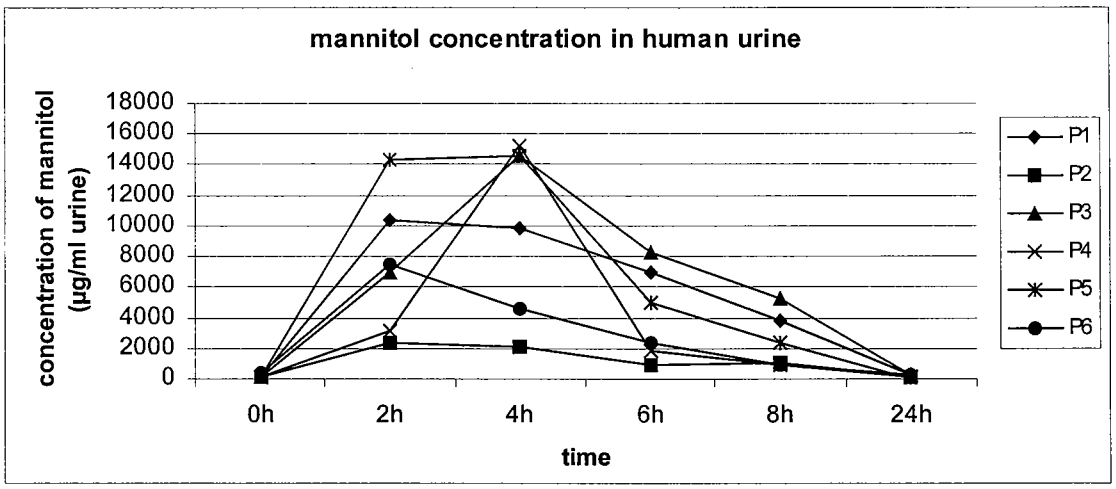

Figure 4: Mannitol concentration in excretion study urine samples of all volunteers (P1-P6)

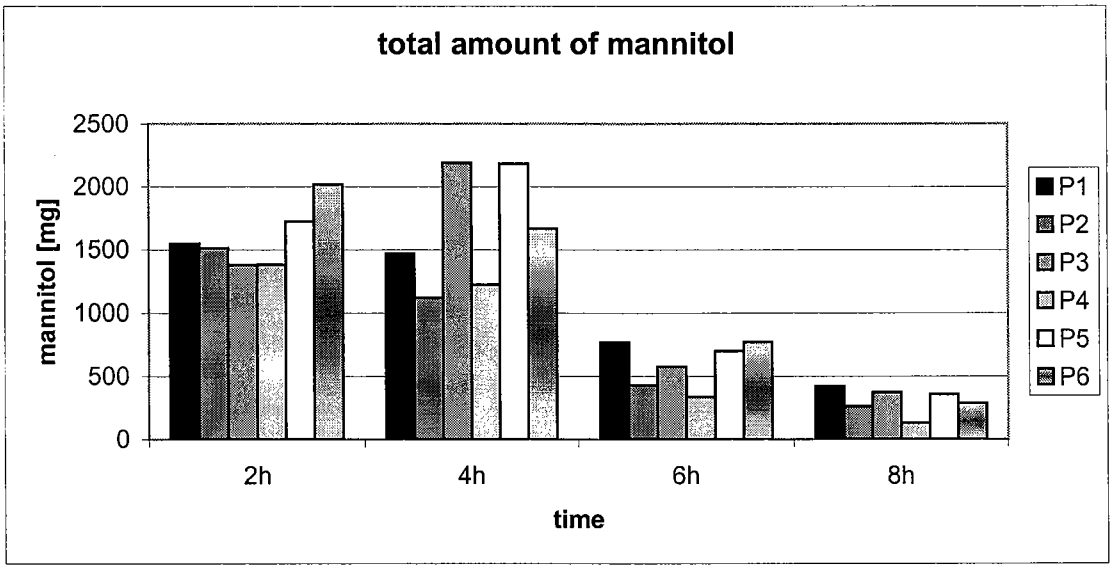

Figure 5: Total amount of mannitol quantified in urine samples of volunteers P1-P6 after 2, 4, 6, and 8h after administration

4. Discussion

Due to the fact that mannitol is poorly intestinally absorbed, high amounts of mannitol were observed in all urine samples. Certainly, an average daily intake of mannitol is much lower than the amount administered in this study, but the fact that mannitol appears in food such as apples, pineapples, asparagus and carrots [2], and owing to its admission as a food additive, high concentrations of mannitol can occur in human urine. The urinary concentration of mannitol after intravenous application was measured with a single sample obtained from a patient treated with dextran and mannitol. Mannitol as a diuretic agent is usually administered as a 15 to 25% solution in a dose of 0.25 to 2 g per kg body-weight over 30 to 60 minutes [5]. Its level was approximately 5 times lower than those found in excretion study urine samples after oral application. These results demonstrate the difficulty to differentiate between orally and intravenously administered mannitol.

References

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