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Stability of 19-norandrosterone- and 19-noretiocholanolone glucuronide in urine under various storage conditions

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INTRODUCTION

It is well established in literature (1-4) that the presence of 19-norandrosterone, a metabolite of nandrolone, in human urine may be the consequence of normal excretion. For this reason the International Olympic Committee (IOC) has fixed cut-off values in urine for 19-norandrosterone. These concentrations are 2 ng/ml for males and 5 ng/ml for females. In the light of this IOC ruling it is therefore important to know if the concentration of 19-norandrosterone glucuronide can decrease during the transportation process of urine samples to the laboratory or during the handling and storage of the samples in the laboratory. If so, this can lead to a false negative result.

AIM

The aim of this study is to determine the stability of 19-norandrosterone and 19-noretiocholanolone glucuronide in human urine under various storage conditions.

METHOD

Four subjects received either a single 100 mg dose of 19-norandrostenediol or 19-norandrostenedione. Fractional urine samples were collected over a period of 5 days. Urine samples obtained from the excretion on day 5 of each product (one sample from each subject) were analysed directly after collection and then stored under various storage conditions.

Short term stability

Urine samples were stored as follows:

20 ml in direct sunlight

20 ml at room temperature (± 22 °C)

20 ml in a refrigerator (± 5 °C)

Samples were analysed after 24 hours (1 day) and 72 hours (3 days).

The pH of all these urine samples was measured prior to analysis.

Long term stability

20 ml aliquots of the urine samples were stored in a freezer at ± -20 °C and then analysed after 1 and 2 months, respectively.

Freeze and thaw stability

20 ml aliquots of the urine samples were stored in a freezer at -20°C for 24 hours (Duplicate samples from each subject). Half of the samples were thawed unassisted at room temperature while the other half were thawed in warm water. When the samples were completely thawed it was transferred back to the freezer and kept frozen for 24 hours. The cycle of thawing and freezing was repeated two more times and then analysed on the third cycle (3 days).

Quantification of 19-norandrosterone and 19-noretiocholanolone were done by using our routine quantification procedure for the two compounds. A calibration curve for each compound was constructed on the day of analysis.

RESULTS

The accuracy of the method is 10% and the concentrations determined in the various samples should therefore not differ by more than 10%. These lines are clearly indicated in each graph.

The pH of the urine samples stored at the different temperatures did not change over the 3 day period.

Short term stability

Fig. 1 and fig. 2 clearly illustrate that both 19-norandrosterone- and 19-noretiocholanolone glucuronide are stable when stored for at least 3 days in direct sunlight, at room temperature and in a refrigerator.

Long term stability

From fig. 3 and 4 it is clear that both 19-norandrosterone- and 19-noretiocholanolone glucuronide are stable for at least 2 months when stored frozen at –20 °C.

Freeze and thaw stability

Freezing and thawing had no influence on the stability (fig. 5 and 6), even if the thawing was done by putting the samples in warm water.

DISCUSSION

Transport and storage of urine samples at high temperatures may lead to bacterial degradation. It is documented (5) that androsterone- and etiocholanolone glucuronide can be converted to 5α-androstandion and 5β-androstandion, respectively, by bacterial activity. Likewise can bacterial activity convert norandrosterone- and noretiochlolanolone glucuronide to 19-nor-5α-androstandion and 19-nor-5β-androstandion, which will cause a decrease in the 19-norandrosterone and 19-noretiocholanolone concentration.

Although these conversions were not measured the results of this study clearly showed that there are no significant decrease in the concentration of 19-norandrosterone or 19-noretiocholanolone when urine containing the glucuronides of these two products were stored under various conditions. It is thus unlikely that the above mentioned conversion did occur.

Usually elevated pH values are the first indication of bacterial activity but the pH values of the urine samples stored at different temperatures, even in direct sunlight were stable indicating that no bacterial activity was present.

This means that if 19-norandrosterone- or 19-noretiocholanolone gucuronide is present in an urine sample from a competitor at a concentration just about the cut-off set by the IOC, it will still be present in this sample at the same concentration when it arrives in the laboratory. Also, the handling and storage of this sample in the laboratory will not influence the concentration. It is thus unlikely that storage and transport at warm conditions will be responsible for a false negative result for 19-norandrosterone. These results are of particular interest if it is requested that the B-sample should be analysed

CONCLUSION

Although it is recommended that urine samples should be stored frozen, it is beneficial to know that the concentration of 19-norandrosterone- and 19-noretiocholanolone glucuronide in urine samples will not be influenced by other storage conditions.

REFERENCES

1. Dehennin L, Bonnaire Y, Plou P, J of Chromatogr B 1999, 721, 301-307
2. Le Bizec B, Monteau F, Gaudin I, Andre F, J of Chromatogr B 1999, 723, 157-172
3. Ciardi M, Ciccoli R, Barbarulo M, Nicoletti R, Koln: Sport und Buch Strauß, 1999, 97-104
4. Saugy M, Robinson N, Cardis C, Schweizer C, Rivier L, Mongin P, Ayotte C, Dvorak J, Koln: Sport and Buch Strauß, 2000, 95-107
5. Geyer H, Schänzer W, Mareck-Engelke U, Donike M, Koln: Sport and Buch Strauß, 1996, 95-114

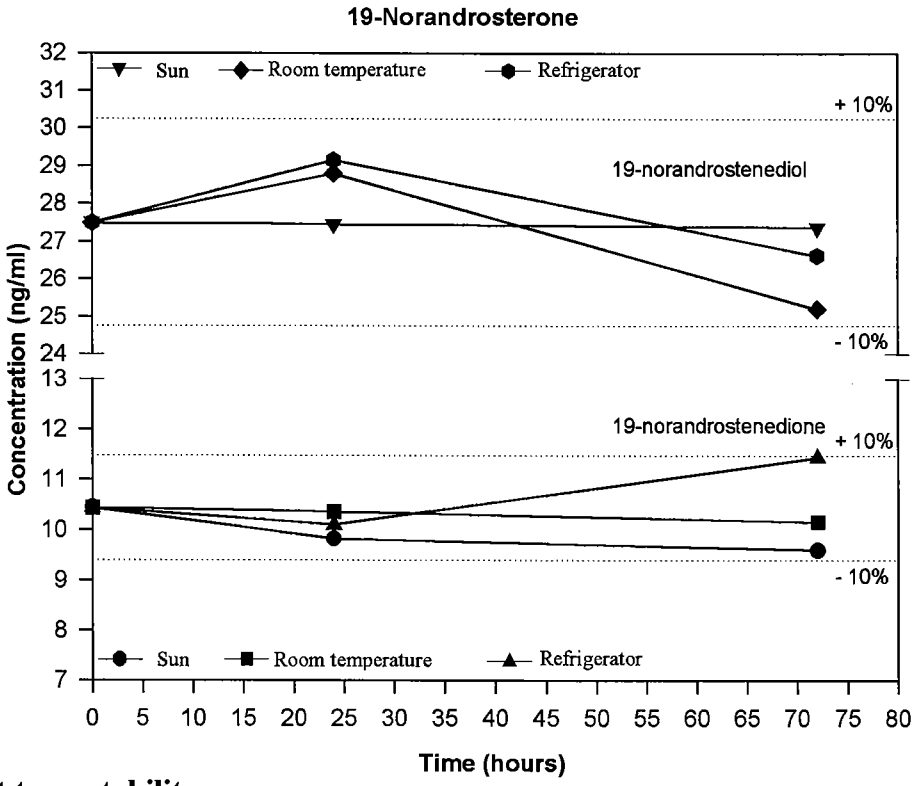

Fig. 1: Short term stability

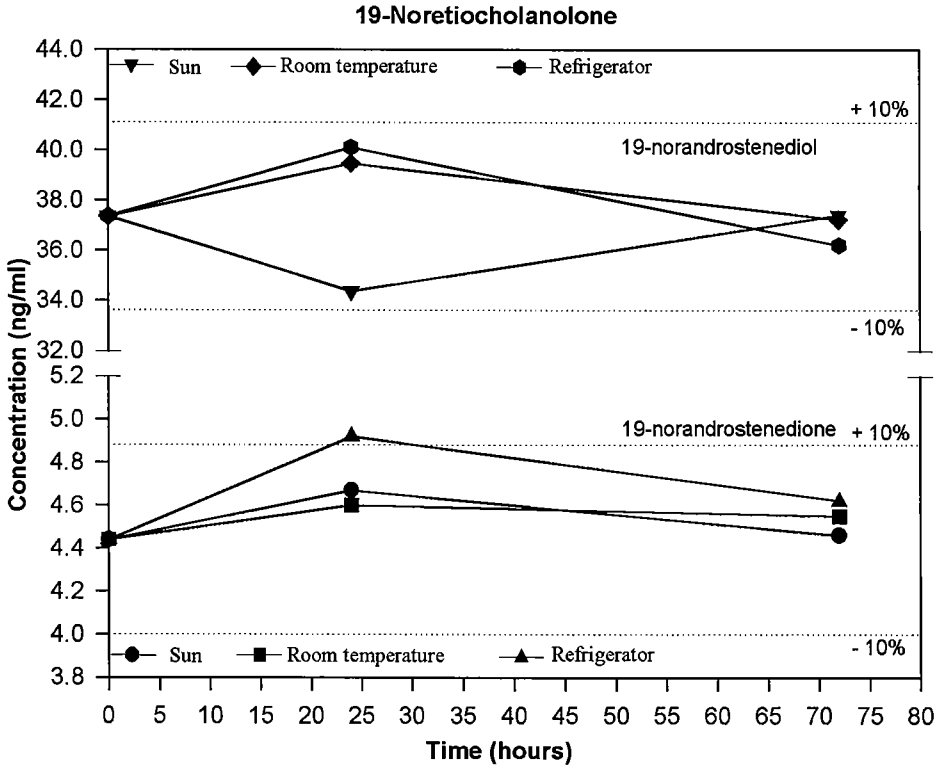

Fig. 2: Short term stability

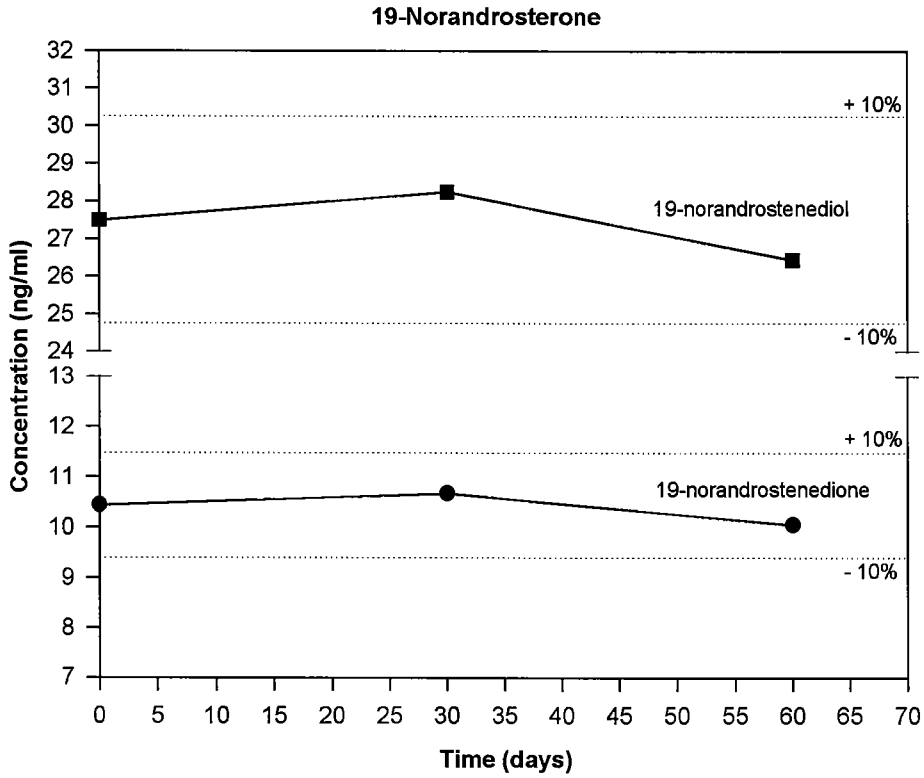

Fig. 3: Long term stability

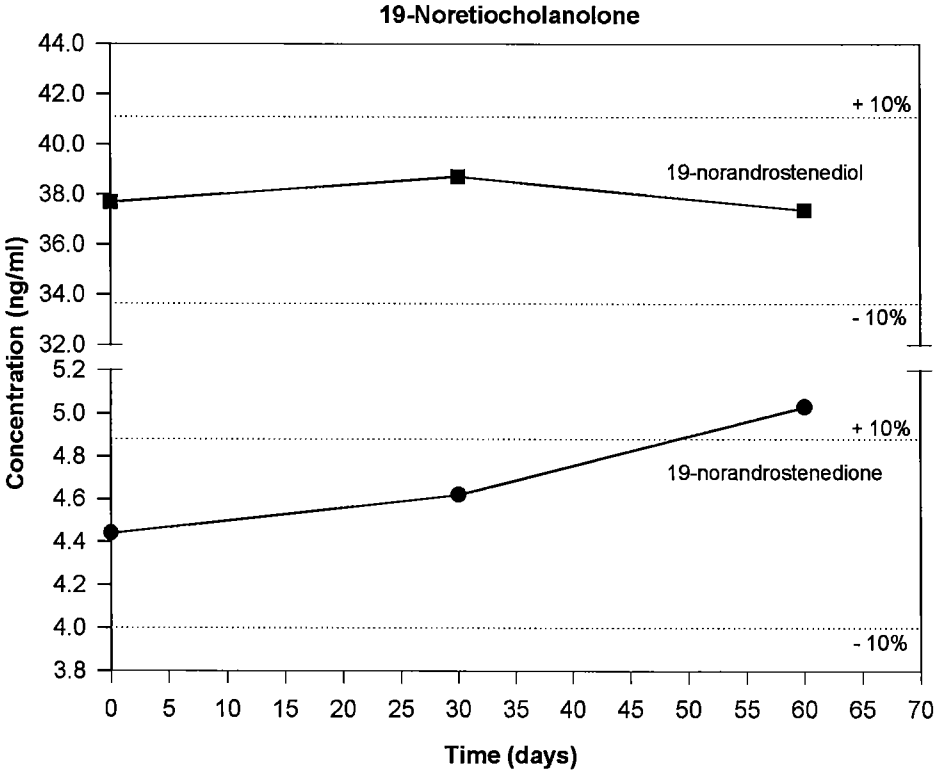

Fig. 4: Long term stability

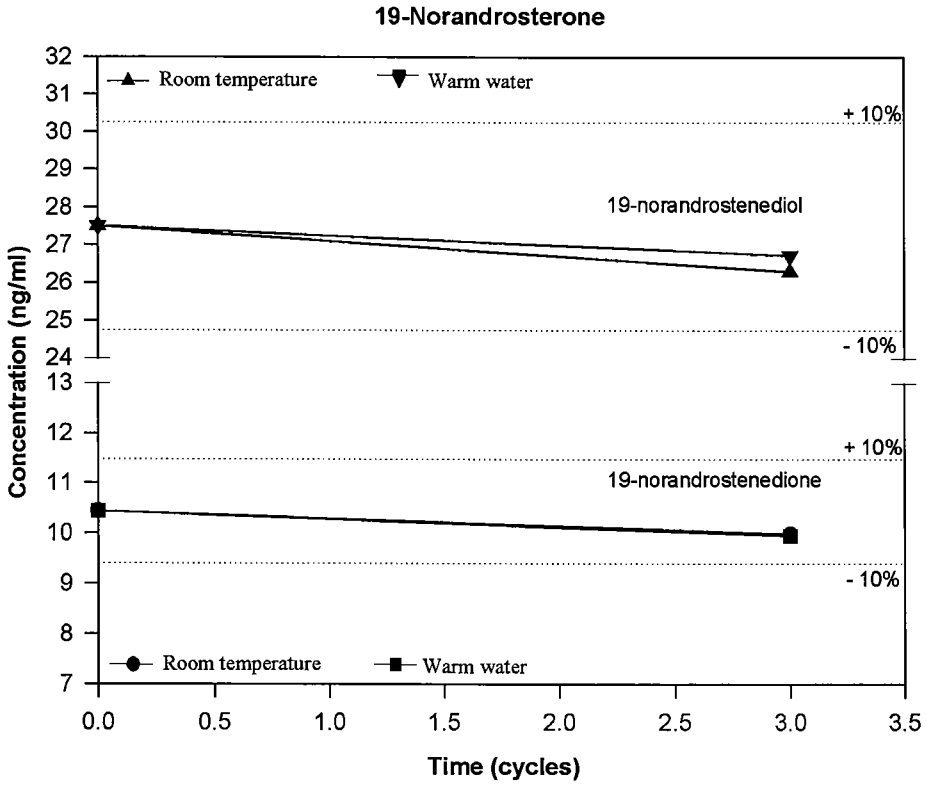

Fig. 5: Freeze-thaw stability

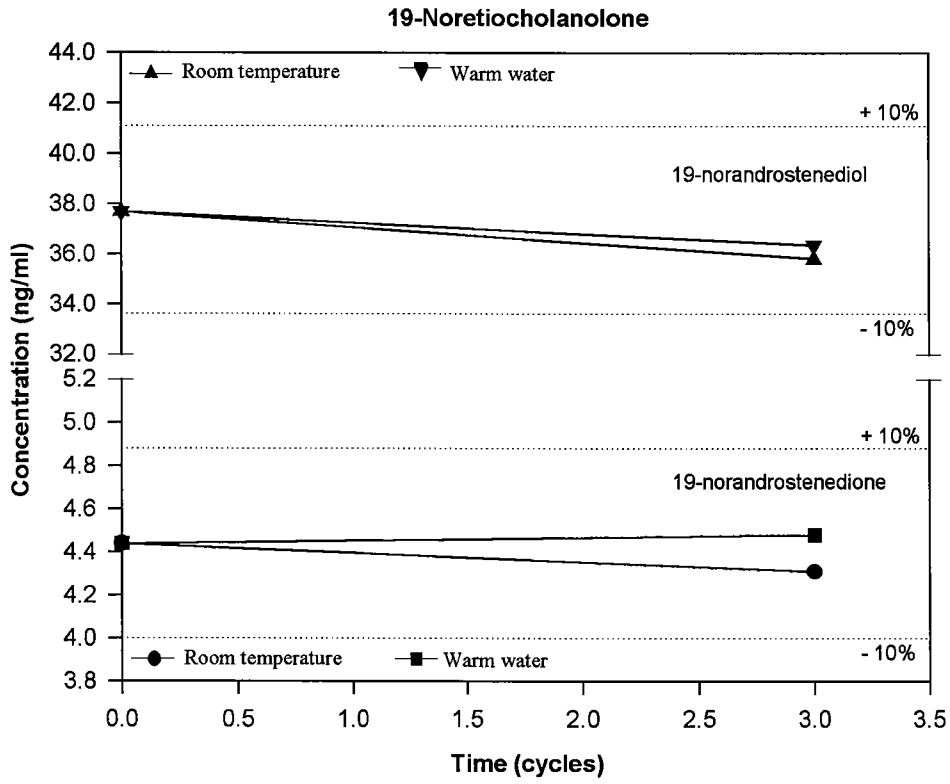

Fig. 6: Freeze-thaw stability