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Stabilization of endogenous steroids in sport urine samples

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Abstract

Former studies have demonstrated the adverse effects of microbial contamination to the endogenous steroid profiles of urine samples collected for doping control purposes. The aim of the current work, funded by WADA, was the implementation of a stabilization method to prevent or minimize urinary steroid degradation, irrespective of the storage or transportation conditions of the samples. For this purpose, a chemical stabilization mixture, consisting of antibiotics, antimycotic substances and protease inhibitors was applied in urine aliquots fortified with conjugated and deuterated steroids and inoculated with nine representative microorganisms. Aliquots with and without the chemical mixture were incubated at 37 °C for 7 days to simulate the transportation period, whereas another series of aliquots were stored at -20 °C to serve as reference. Microbial growth was assessed immediately after inoculation and at the end of the incubation period. Variations in pH and specific gravity values were recorded. Gas chromatography–mass spectrometry (GC-MS) analysis was performed for the detection of steroids in the free, glucuronide and sulfate fractions. Evaluation of results showed that the chemical mixture had a lethal effect on the nine microorganisms tested. The pH and sg values of the urine samples were not significantly affected by the addition of the chemical mixture. Hydrolysis of glucuronide and sulfate conjugates, formation of metabolic by-products, and production of boldenone induced by certain microorganisms were prevented in the presence of the chemical mixture.

References

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