"Optimization of the synthesis of the chemical stabilization mixture of urine samples with simultaneous minimization of analytical matrix interferences."

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Aims

The doping control samples are not protected by environment conditions during their transportation from the collection sites to the WADA Accredited Laboratories. The WADA Technical Document for reporting doping violations concerning endogenous steroids is issuing parameters to check for urinary microbial integrity for certain steroids. Endogenous steroids profiles of urine samples may undergo changes due to the occurrence of microorganisms that can be found in the human body or the surrounding environment, especially during their transportation in the warm periods of the year. Moreover, suspicions of tampering of doping control samples with proteases to mask the administration of peptide hormones were confirmed recently.

The Doping Control Laboratory of Athens, OAKA, was granted in 2005 by a WADA research fund to study different approaches regarding stabilization of urine doping control samples during their transportation to the Laboratories. Evaluation of results showed that the application of the stabilization mixture into urine aliquots had a lethal effect on the populations of the microorganisms tested. Moreover, when the stabilization mixture was included in urine aliquots, enzymatic digestion of rEPO and dissociation of the hCG intact molecule in the presence of proteases were inhibited. However, matrix interferences were recorded in mass spectrometric routine analytical procedures.

The current project focuses on the creation of a specially designed urine sample collection plastic container, coated in its interior surface with a suitable chemical stabilization mixture aiming at improving the quality of sport urine samples. The stabilization mixture should be effective in prohibiting degradation caused by microorganisms and proteolytic enzymes, have immediate efficiency, low cost, lack of toxicity and absence of matrix interferences in the doping control analysis. In the current study, the different components of the stabilization mixture will undergo experimental cycles trying to optimize and compromise between stabilization efficiency and analytical matrix interferences.

Results and Conclusions

The current project focuses on the creation of a specially designed plastic urine collection container, coated in its interior surface with the previously developed in-house stabilization mixture aiming at improving the quality of sport urine samples without posing analytical interferences problems in accredited laboratories. Before implementing the chemical stabilization mixture in an industrial scale, the right application form (liquid, freeze-dried, or spray-coated) should be carefully selected and tested in pilot-scale so that it can be implemented in the doping control sampling protocol. The spray coating application form was selected as the more easily acceptable by the end user.

The chemical stabilization mixture was spray coated in the interior walls of plastic urine Collection containers to simulate the doping control urine collection process. Its efficiency against steroid glucuronide degradation caused by five microorganisms (E. coli, N. simplex. E. faecalis, A. flavus, C. albicans) and enzymatic breakdown of intact hCG induced by six proteolytic enzymes (papain, pepsin, trypsin, α-chymotrypsin, bromelain and subtilisin A) was investigated during incubation experiments at 37 ºC. Also, two WADA accredited laboratories, DoCoLab (Ghent) and Laboratorio Antidoping FMSI (Rome) undertook the investigation of the stabilization mixture efficiency against rEPO degradation in the presence of proteolytic enzymes. Moreover, a systematic evaluation of eventual analytical interferences in the presence of the stabilization mixture in spray coated form was conducted by the Athens Doping Control Lab (DCLA), DoCoLab and Laboratorio Antidoping FMSI.

The addition of the chemical stabilization mixture in spray-coated form in the interior surface of plastic urine containers inhibited microbial growth and prevented steroid degradation at the end of a 7-day incubation period at 37 °C. The occurrence of the chemical stabilization mixture in spray-coated form prevented rEPO degradation by four proteases (papain, pepsin, trypsin and bromelain) during the 4-day period at room temperature. When α-chymotrypsin or subtilisin A were spiked, IEF signals were detectable at t = 0 in the basic area of the gel only in stabilized aliquots but were eliminated at t = 4 in both the stabilized and unstabilized aliquots. Regarding the degradation of intact hCG induced by proteolytic enzymes, hCG levels were higher in stabilized aliquots at the end of the 4-day incubation period at 37 °C for five out of six proteases tested (bromelain, papain, pepsin, α-chymotrypsin and trypsin). The presence of the chemical stabilization mixture in spray-coated form slowed down the degradation of intact hCG when subtilisin A was spiked but at t = 4, intact hCG levels were undetectable, irrespective of the presence or absence of the stabilization mixture.
The evaluation of analytical interferences in the presence of the stabilization mixture in spray coated form, conducted by three WADA accredited labs, showed that some volatile compounds were negatively affected, depending on the urine matrix.