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THE POTENTIAL OF BIOASSAYS AND TIME-OF-FLIGHT MASS SPECTROMETRY IN URINE TESTING FOR BANNED AND DESIGNER STEROIDS

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New anabolic steroids show up occasionally in sports doping and in veterinary control. The discovery of these designer steroids is facilitated by findings of illicit preparations, thus allowing bioactivity testing, structure elucidation using NMR and mass spectrometry, and final incorporation in urine testing. However, as long as these preparations remain undiscovered, new designer steroids are not screened for in routine sports doping since the established GC/MS and LC/MS/MS methods are set-up to the monitoring of a few selected ions or MS/MS transitions of known substances only. In this study, the feasibility of bioactivity testing and mass spectrometric identification is being investigated for trace analysis of designer steroids in urine. Following enzymatic deconjugation and a generic solid phase extraction the samples are analyzed by gradient liquid chromatography (LC) with effluent splitting towards two identical 96-well fraction collectors. One well plate is used for androgen bioactivity detection using a robust yeast reporter gene bioassay yielding a biogram featuring a 20 second time resolution. The bioactive wells direct the identification efforts to the corresponding well numbers in the duplicate plate. These are subjected to high resolution LC using a short column packed with 1.7 μm C_{18} material and coupled with electrospray quadrupole time-of-flight mass spectrometry (LC/QTOFMS) with accurate mass measurement. Element compositions are calculated and used to interrogate electronic substance databases. The feasibility of this approach is demonstrated via the screening of human urine samples spiked with the designer anabolic steroid tetrahydrogestrinone (THG).

Full details will be published elsewhere:
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