"Novel Approach to detect and identify low abundant long-term metabolites of prohibited drugs using GC/C/IRMS and HRMS"

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Project Overview

Most substances relevant to doping controls undergo considerable metabolism following administration. The generated metabolites are commonly less toxic and more polar which allows for renal clearance. Consequently, in urine the analysis of metabolites is more promising than attempts to detect the ingested or injected compound, particularly when these metabolites are excreted for a longer period of time than the drug. These so-called long-term metabolites are of great interest especially for those doping agents that are predominantly used during out-of-competition periods as for instance in case of anabolic androgenic steroids.

In order to identify drug metabolites in biological matrices, stable isotope labeling of compounds has proven to be a helpful means within the last 3 decades. Usually, hydrogen is replaced by its heavier isotope deuterium at 3 to 5 locations within the steroid nucleus and the resulting mass shift was detected by means of conventional mass spectrometers. The sensitivity of this approach has recently been considerably improved by using hydrogen isotope ratio (HIR) mass spectrometry instead of conventional mass spectrometers as these HIR-dedicated systems are built to determine the deuterium/hydrogen ratio at natural abundance, i.e. with approximately 1 out of 10000 hydrogen atoms representing a deuterium atom. For deuterium-labeled compounds commonly up to 10 % of hydrogen is substituted and thus enables a highly sensitive detection of any metabolite of the administered compound as long as it carries the deuterium-label. This feature will be exploited in a novel approach to investigate the metabolism of clenbuterol and nandrolone. Both substances are in the scope of sports drug testing since several years and the systematic study of their metabolism will be conducted to support their long-term detection and determination of respective sources (i.e. endogenous/natural or artificial/contamination).

Results and Conclusions:

Employing stable isotope-labeled drugs and GC/IRMS for metabolism studies significantly facilitates the detection of relevant analytes also at lowest abundance in complex matrices such as human urine. In the present research project, the metabolism of two critical anabolic agents, 19-nortestosterone and clenbuterol, was revisited utilizing the recently established approach. Triply deuterated 19-nortestosterone and nine-fold deuterated clenbuterol was used to characterize the drugs' metabolic profile and to complement and/or corroborate the picture of long-term metabolites as well as the possibilities to differentiate the origin of analytes. In case of

19-nortestosterone, metabolites supporting the classification of low abundant 19-norandrosterone findings as of natural or xenobiotic nature is desirable; in case of clenbuterol, information facilitating the differentiation of contaminated food ingestion from deliberate drug misuse is needed.

The results obtained for both drugs were manifold and a substantial number of known and several yet unknown metabolites as well as sample preparation-derived artefacts were identified. A new 19-nortestosterone metabolite was observed, which was traceable as long as 19-norandrosterone in human urine in the conducted elimination study. The structure of this compound is not entirely clarified yet, and since it appeared to exhibit significant interindividual variability (as suggested by means of routine doping control samples and confirmed adverse analytical findings), its utility for sports drug testing purposes might unfortunately be rather limited. Also for clenbuterol, metabolites were detected and attributed to oxygenation products of the drug. As these are expected to possess different elimination kinetics than clenbuterol itself, a means to differentiate the recent intake of a minute amount (i.e. via food contamination) from remnants of clenbuterol being eliminated from drug abuse weeks ago could be obtained. Here, urinary clenbuterol / metabolite ratios need to be determined in future studies.