"Probing for new long-term metabolites of trenbolone by hydrogen isotope ration mass spectrometry"

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Project Overview

Trenbolone is a veterinary product marketed as an effective growth promoter in animal husbandry and has never obtained medical approval for humans. Nevertheless, it is presumed to be regularly misused by athletes due to its performance enhancing properties and resulted in various adverse analytical findings in the recent years. By the end of 2016, the issue of trenbolone in sports was particularly emphasized by the McLaren report as one of the anabolic steroids extensively misused in Russia. The cocktail administered to athletes consisted of oxandrolone, methenolone and trenbolone. While the first 2 steroids have been carefully investigated in recent years and long-term metabolites were identified, this research is still pending for trenbolone. Since more than 25 years doping control laboratories search for trenbolone itself and its major urinary metabolite epitrenbolone. Detectability of trenbolone was only improved by using liquid chromatography-mass spectrometry instead of gas chromatography-mass spectrometry.

Aim of this study will be the in-depth investigation of the trenbolone metabolism in humans using a recently established methodology developed especially for metabolite detection in sports drug testing. After administration of deuterium-labeled trenbolone, all metabolites carrying this label will be identified unambiguously by hydrogen isotope ratio mass spectrometry. Then all analytes of interest will be quantified and identified by means of high-resolution and high-accuracy mass spectrometry. Within this study we will focus on the detection of long-term metabolites prolonging the retrospective detection of trenbolone misuse.

Results and Conclusions:

Trenbolone is a synthetic anabolic-androgenic steroid, which has been misused for performance enhancement in sports. The detection of trenbolone doping in routine sports drug testing programs is complex as methods utilizing gas chromatography/ mass spectrometry are complicated by unspecific derivatization products and artefacts, and liquid chromatography/ mass spectrometry-based assays have shown to allow for comparably high limits-of-detection only. The number of previously reported metabolites in human urine is limited, and most analytical methods rely on targeting epitrenbolone, trenbolone glucuronide, and epitrenbolone glucuronide.

In order to probe for the presence of additional trenbolone metabolites and to re-investigate the metabolism, an elimination study was conducted. One single dose of 10 mg of 5-fold deuterated trenbolone was administered to a healthy male volunteer and urine samples were collected for 30 days. For sample processing, published protocols were combined considering

unconjugated, glucuronic acid-, sulfo- and alkaline-labile conjugated steroid metabolites. The sample preparation strategy consisted of solid-phase extractions, liquid-liquid extractions, metabolite de-conjugation, HPLC fractionation, and derivatization. Analytical methods included gas chromatography/ thermal conversion/ hydrogen isotope ratio mass spectrometry combined with single quadrupole mass spectrometry as well as liquid chromatography/ high accuracy/ high resolution mass spectrometry of the hydrolyzed and non-hydrolyzed samples.

Twenty deuterium-labelled metabolites were identified including glucuronic acid-, sulfo- and potential cysteine-conjugates, and characterized by parallel reaction monitoring experiments yielding corresponding product ion mass spectra. Main metabolites were attributed to trenbolone-diol and potential trenbolone-diketone derivatives excreted as glucuronic acid and sulfo-conjugated analytes with detection windows of 5 respectively 6 days. Further characterization was conducted with pseudo MS^3 experiments of the intact conjugates and by comparison of resulting product ion mass spectra with reference material.