"Detection and characterization of new long-term steroid metabolites by MRM GC-CI-MS/MS."

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Project Overview

The identification of anabolic androgenic steroids (AAS) is a vital issue in doping control. Due to the performance enhancing properties of AAS, the World Anti-Doping Association (WADA) banned their use but according to the annual report of WADA, steroids are still very popular amongst athletes and are responsible for half of all adverse analytical findings. The search for metabolites with longer detection times remains an important task and the introduction of new long-term metabolites for exogenous AAS such as for example stanozolol, methanedione and dehydrochloromethyltestosterone, led to a 4 - 80-fold increase of adverse analytical findings due to the prolonged detection time.

This project aims at finding new long-term metabolites for a number of AAS by application of our newly developed gas chromatography chemical ionization triple quadrupole mass spectrometry (GC-CI-MS/MS) protocol for metabolite detection and identification. Chemical ionization in combination with triple quadruple technology has proven to significantly increase the sensitivity for a wide range of compounds in comparison with electron impact (EI). In addition, GC-CI-MS/MS is characterized by AAS structure correlated fragmentation pathways. The combination of both factors allows the set up of a sensitive MRM method, designed to find previously unknown but expected metabolites by selection of theoretical transitions for expected metabolites.

Results and Conclusions:

In 2015, a new GC triple quadrupole MS method that used chemical ionization (CI), instead of EI was introduced. This new GC-CI-MS/MS method opened new possibilities in the search for new metabolites as CI is a soft ionization. The correlations between fragmentation behavior and AAS structure could be revealed and fragmentation pathways have been postulated. This enabled the search for previously unknown but expected metabolites by selection of their predicted transitions. The aim of the current project was to set up an efficient approach for searching new metabolites by application of these newly discovered structure depended fragmentation pathways and to find new long-term metabolites. The following AAS were selected: drostanolone, metenolone, mesterolone, oxymesterone, formebolone and methyltestosterone.

Novel long-term metabolites for oxymesterone and mesterolone were detected and characterized. This demonstrates that GC-CI-MS/MS is capable of detecting (and characterizing) metabolites that can be missed with other (more frequently) used techniques. For oxymesterone, the metabolite was

identified as 18-nor-17β-hydroxymethyl-17α-methyl-4-hydroxy-androst-4,13-diene-3-one. It is primarily excreted as a glucuronide. For mesterolone, the metabolite was identified as 1α-methyl-5α-androstan-3,6,16-triol-17-one and its sulfate form resulted in a prolonged detection time for mesterolone abuse.

For metenolone, a metabolite, primarily excreted as sulfate, was found to have a slightly improved detection window in comparison with the currently monitored metabolites. Likely, the metabolite is 1β-methyl-5α-androstan-17-one-3ζ-sulphate and for the first time it is documented to provide the longest detection time.

In general, this study illustrates that sulfated steroids are becoming increasingly important for doping control analyses as they allow longer detection times for AAS and can provide valuable information.

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