

## PROJECT REVIEW

### **“Development of methods for the detection of the misuse of the aromatase inhibitors anastrozole, letrozole and vorozole in urine”**

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As notified by the IOC and WADA on June 1, 2001 the use of aromatase inhibitors is prohibited for male athletes beginning September 1, 2001. Aromatase inhibitors are listed in the class of peptide hormones, mimetics and analogues. Three classes of aromatase inhibitors are differentiated:

1. aminoglutethimide
2. non steroidal aromatase inhibitors (anastrozole, letrozole, vorozole)
3. steroidal aromatase inhibitors (exemestane, formestane)

Actually there exists only a mass spectrometric method for the detection of the misuse of aminoglutethimide in urine

The aim of the study is to develop mass spectrometric methods for the detection of the misuse of the non steroidal aromatase inhibitors anastrozole, letrozole and vorozole in urine. If possible the methods should be comprehensive methods for the simultaneous detection of several aromatase inhibitors and should be added to existing screening procedures of doping analysis.

The project includes investigations of the metabolism of the aromatase inhibitors, development of methods for suitable sample preparation and derivatisation, choice of the suitable mass spectrometric method (GC/MS; LC/MS) and validation of the methods. For the investigation urines of female patients treated with non steroidal aromatase inhibitors will be used.

## **Development of methods for the detection of the misuse of the aromatase inhibitors anastrozole and letrozole in urine**

### **Results and conclusions**

The methodology used for the detection of the misuse of the aromatase inhibitors anastrozole and letrozole could be incorporated into the existing screening procedures for doping substances.

An inclosure of anastrozole parent compound into the screening procedure for selected anabolic androgenic steroids [1] via LC-MS/MS is recommended. The fragmentation pattern shows suitable ion transitions at  $m/z$  294/225, 294/210, 294/142 and 294/130. The characterization of the method shows a linear and homoskedastic calibration curve with a detection limit of 0.02 ng anastrozole per milliliter as well as high accuracy and precision. The standard operating procedure for selected anabolic androgenic steroids via LC-MS/MS is a robust screening method with a recovery of 97% for anastrozole. [2] \*

For the detection of letrozole misuse screening for the letrozole metabolite bis-(4-cyanophenyl)-methanol by GC-MS is an excellent tool. The EI-MS fragmentation pattern of the TMS derivative shows suitable ions with high intensity [ $m/z$  217 ( $M^+$ -89 / base peak),  $m/z$  291 ( $M^+$ -15) and  $m/z$  306 ( $M^+$ )]. The validation of the method shows a linear and homoskedastic calibration curve with an estimated lower limit of detection of 4.4 ng/ml. The standard operating procedure for anabolic steroids [1,3] is a robust screening method with a recovery of 90% for bis-(4-cyanophenyl)-methanol. [4]

### **Publications and poster presentations**

- [1] Mareck U, Thevis M, Guddat S, Gotzmann A, Bredehöft M, Geyer H, Schänzer W: Comprehensive sample preparation for anabolic steroids, glucocorticosteroids, beta-receptor blocking agents, selected anabolic androgenic steroids and buprenorphine in human urine. In: Schänzer et al (Eds.) Recent advances in doping analysis (12) Sport und Buch Strauß, Köln, 2004, 65-68.
  - [2] Mareck U, Geyer H, Guddat S, Haenelt N, Koch A, Opfermann G, Thevis M, Schänzer W: Screening for anastrozole in doping analysis. In: Schänzer et al (Eds.) Recent advances in doping analysis (13) Sport und Buch Stauß, Köln, 2005 (accepted for publication).
  - [3] Geyer H, Schänzer W, Mareck-Engelke U, Nolteernsting E, Opfermann G: Screening procedure for anabolic steroids – the control of the hydrolysis with deuterated androsterone glucuronide and studies with direct hydrolysis. In: Schänzer et al (Eds.) Recent advances in doping analysis (5) Sport und Buch Strauß, Köln, 1998, 99-101.
  - [4] Mareck U, Sigmund G, Opfermann G, Geyer H, Schänzer W: Screening on letrozole and its metabolite in doping analysis. In: Schänzer et al (Eds.) Recent advances in doping analysis (12) Sport und Buch Strauß, Köln, 2004, 203-209.
- \* The results were orally presented at the 23<sup>rd</sup> Cologne Workshop on Dope Analysis (27.02. - 04.03.2005).