

## **“Improving the Traceability of Steroid Abuse by Introduction of 2H/1H-Analysis of Urinary Steroids”**

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### **PROJECT REVIEW**

Stable isotope techniques are successfully employed to detect doping with synthetic steroid hormones such as testosterone or its precursors. The test exploits the fact that synthetic testosterone exhibits a different ratio of the stable carbon isotopes  $^{13}\text{C}$  and  $^{12}\text{C}$  compared to its natural counterpart. However, the  $^{13}\text{C}/^{12}\text{C}$  ratio is also influenced by diet and other factors. In some regions the natural  $^{13}\text{C}/^{12}\text{C}$ -ratio of steroid hormones is close to that of synthetic material. This is due to the  $^{13}\text{C}/^{12}\text{C}$ -ratios of the diet. Testosterone doping thus can go undetected under these circumstances.

The other element present in all steroids is hydrogen. Like carbon it has two stable isotopes,  $^1\text{H}$  and  $^2\text{H}$ . The  $^2\text{H}/^1\text{H}$ -ratio is probably better suited to discriminate between synthetic and natural testosterone. Especially when the  $^{13}\text{C}/^{12}\text{C}$ -test fails it can be expected that the  $^2\text{H}/^1\text{H}$ -ratio can still betray the presence of synthetic steroids.

### **Results and Conclusions**

Stable carbon isotope analysis of endogenous steroids is a well-established method to demonstrate the illicit administration of synthetic steroids. The latter typically feature lower  $^{13}\text{C}/^{12}\text{C}$  ratios than their biological congeners. Testosterone, the principal male sex hormone, plays a pivotal role here. Abuse still is frequent but major progress in the fight against doping has been achieved by analysing the  $^{13}\text{C}/^{12}\text{C}$  ratios of testosterone and of its major metabolites.

The methodology obviously requires that the  $^{13}\text{C}/^{12}\text{C}$  ratios of the synthetic material sufficiently differ from the biological baseline. Due to variability in the composition of the diet, this not always the case. In addition, black-market testosterone preparations have been found which exhibit inconspicuous carbon isotope signatures.

There is another so-called isotope system which may be useful here. The isotope ratios of hydrogen ( $^2\text{H}/^1\text{H}$ ) typically even exhibit much stronger variation than those of carbon. In fact, it has been demonstrated that synthetic steroids tend to be  $^2\text{H}$  enriched vs. corresponding biological compounds.

The methodology is, however, much more delicate. This is mostly due to the small abundance of  $^2\text{H}$  (merely ca. 0.0015 %). Consequently, much more material is required than for  $^{13}\text{C}/^{12}\text{C}$  analysis. This significantly compromises the applicability of  $^2\text{H}/^1\text{H}$  analysis in sports drug testing. Moreover, at low abundances significant and unacceptable bias will be present in the apparent  $^2\text{H}/^1\text{H}$  ratios when signal intensities are too low.

Therefore, as a priority, the focus of this research project was to improve the analytical method first in order to render  $^2\text{H}/^1\text{H}$  analysis fit for the requirements of sports drug testing.

The conversion of organic material to hydrogen is a mandatory pre-condition for  $^2\text{H}/^1\text{H}$  analysis. This is achieved by so-called high temperature conversion at ca 1440°C in reducing environments. The process seems to be sensitive to competing reactions associated by isotope fractionation. This will be less pronounced at higher abundances of the relevant educts.

For these and other reasons, we hypothesized that presence of additional sources of hydrogen would mitigate these problems. A device was designed which allows to evaporate organic solvents and to feed them into the reactor.

Significant improvements could be achieved by this approach. The required amounts of steroids for valid  $^2\text{H}/^1\text{H}$  analysis could roughly be reduced by 50 %. While some refinement still is required, this fundamentally renders  $^2\text{H}/^1\text{H}$  analysis of steroids a feasible additional option to counteract abuse of synthetic steroids.