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## ***In vitro* and *in vivo* metabolism studies of a nutritional supplement containing methylstenbolone**

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### **Abstract**

Ethical objections often limit the use of human volunteers for excretion studies with designer drugs. Metabolism studies are however essential to find the best markers of doping agents. Alternative models can therefore be a valuable tool for anti-doping laboratories to identify metabolites of new substances for inclusion in existing screening methods.

Recently, a 'nutritional' supplement, named 'Ultradrol', was introduced on the market. This product contains, according to the label, 17 $\alpha$ -methylstenbolone. Nevertheless, analysis of the content revealed the presence of both methylstenbolone and methasterone, a structurally closely related steroid. To elucidate the phase I metabolism of this steroid product an uPA<sup>+/+</sup>-SCID chimeric mouse model and human liver microsomes (HLM) were used. Methylstenbolone was isolated from the supplement via HPLC fraction collection and administered to both models. By using HLM ten mono-hydroxylated metabolites (U1-U10) and a still unidentified derivative of methylstenbolone (U13) were detected. In the chimeric mouse urine only di-hydroxylated methylstenbolone derivatives (U11-U12) were identified. Neither methasterone nor its metabolites were detected after administration of methylstenbolone. Administration of the steroid product to both models resulted mainly in the detection of methasterone metabolites and these were very similar to those already described in literature.

An MRM method on GC-triple quadrupole MS was developed to detect misuse of methylstenbolone. In a sample, previously tested positive for methasterone, methylstenbolone and U13 were additionally detected, indicating the use and the applicability of the developed method.

The results of this study will be published elsewhere.