# *“Ecdysterone as Non-Conventional Anabolic Agent, Part 2: Urinary Excretion, Metabolism and Prevalence in Elite Athletes”*

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## Project Overview

Increasing numbers of dietary supplements with ecdysteroids are marketed as “natural anabolic agents”. Part 1 of the project demonstrated the performance enhancement of an ecdysterone supplementation in combination with resistance training in humans. To allow for detection of an ecdysterone administration in doping control, the metabolism of ecdysterone and its urinary excretion will be investigated. For method development additional in vitro experiments will be conducted to help targeting the right potential metabolites. Using the prospected metabolites, a targeted method will be used to analyse post-administration urines. This approach will be complemented by non-targeted analyses using high-resolution mass spectrometry. Finally, the project aims in providing a method useful for screening of ecdysterone and its metabolites in compliance with existing screening procedures in anti-doping laboratories.

Using these results the prevalence of ecdysterone in samples from human sports anti-doping control from different regions will be monitored.

## Results and Conclusions:

Ecdysterone is a phytosteroid widely discussed for its various pharmacological, growth-promoting, and anabolic effects, mediated by the activation of estrogen receptor beta (ERbeta). Performance-enhancement in sports was demonstrated recently, and, in 2020, ecdysterone was consequently included in the Monitoring Program of the World Anti-Doping Agency to detect potential patterns of misuse in sport. Dietary supplements containing ecdysterone were analyzed for their quality. Assay revealed to be poor for the majority of the products.

Only a few studies on the pharmacokinetics of ecdysterone in humans have been reported so far. In this study, a single oral dose of 50 mg of ecdysterone was administered to ten volunteers (five males, five females). After a washout period, two out of the female volunteers were tested a second time. Analysis of serum samples was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) after solid-phase extraction. Kinetic parameters were determined based on this data. Additionally, post-administration urine samples were analyzed using dilute-and-inject LC-MS/MS. Identification and quantitation of ecdysterone and of two metabolites, 14-deoxy-ecdysterone and 14-deoxy-poststerone, were achieved. Ecdysterone was the most abundant analyte present in post-administration urine samples, detected for more than two days, with a maximum concentration (Cmax) in the 2.8–8.5 h urine (Cmax = 4.4–30.0 µg/mL). The metabolites 14-deoxy-ecdysterone and 14-deoxy-poststerone were detected later, reaching the maximum concentrations at 8.5–39.5 h (Cmax = 0.1–6.0 µg/mL) and 23.3–41.3 h (Cmax = 0.1–1.5 µg/mL), respectively. Sex-specific differences were not observed. Cumulative urinary excretion yielded average values of 18%, 2.3%, and 1.5% for ecdysterone, 14-deoxy-ecdysterone, and 14-deoxy-poststerone, respectively. Ecdysterone and 14-deoxy-ecdysterone were excreted following first-order kinetics with half-lives calculated with three hours, while pharmacokinetics of 14-deoxy-poststerone needs further evaluation.

Due to the potential generation of metabolites by gut bacteria that may cause significant variations in the metabolic profile, an integration of further isomers and analogues may also be appropriate. Analytical properties of ecdysterone and its metabolites were evaluated to assess the possibility of integrating them into existing procedures currently used for screening in anti-doping laboratories. Ecdysterone and its metabolites may be easily integrated into current initial testing procedures (ITP) for monitoring the prevalence in elite sports. LC-MS/MS showed excellent eligibility for these analytes. Alternatively, GC-MS analysis is possible after TMS derivatization. If extraction is required or desired, SPE was found to be by far superior to LLE. Enzymatic hydrolysis did not provide advantages over the analysis of the unconjugated fraction only.

Out of 1100 doping control samples 2% revealed positive findings for ecdysterone. The samples were collected from athletes performing a broad variety of sports. Up to now, no classification of “high prevalence” type of sports is seen. Compared with the concentrations found after oral administration of 50 mg of ecdysterone, their concentrations were rather low.