

PROJECT REVIEW

“Degradation of urine samples and its influence on the $^{13}\text{C}/^{12}\text{C}$ ratios of excreted steroids”

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The determination of carbon isotope ratios (CIR) in order to detect the misuse of endogenously occurring steroids is nowadays a routine method in doping control. The comparison of the isotope ratios of androgenic steroids or their metabolites with steroids derived from other metabolic pathways allows for clear discrimination between an endogenous and an exogenous, i.e. administered, androgenic steroid.

Since the 1990's the microbial degradation of urine samples stored under inappropriate conditions is well investigated. Many microorganisms could be identified and their specific action on steroids and steroid conjugates was ascertained. The main chemical conversions are hydrolysis of gluco- or sulpho-conjugates and dehydrogenation of hydroxyl-groups and the steroid backbone.

The impact of these chemical transformations on the steroid profile with its concentration thresholds and diagnostic ratios is described in literature but no data at all is at hand for possible changes in the CIR of different steroids due to microbial degradation. A change in the $^{13}\text{C}/^{12}\text{C}$ ratios of urinary steroids due to degradation might impede the use of this technique in doping control analysis.

Therefore, the stability of CIR for different selected steroids and their degradation products will be investigated in urines stored at 37°C and the impact of these storing conditions on the validity of CIR measurements will be investigated.

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Results and Conclusion:

The influence on CIR of different urinary steroids and steroid-conjugates during degradation was investigated. Regarding glucuronidated steroids which are used in doping control analysis, no significant influence on CIR during the first weeks of the study could be detected. This changed with emerging of the dehydrogenation products 4DN, ADN and EDN and therefore suggested careful interpretation of CIR results in samples showing these strong indications of degradation. The same applied for steroids excreted as sulfates. Especially at the beginning, unconjugated steroids showed strongly depleted $\delta^{13}\text{C}$ values and therefore shall not be utilised in doping control analysis. The reasons for this strong fractionation could not be identified unambiguously within this study and further research on the deconjugation of steroid glucuronides seems advisable.

The results obtained for DCM and DHEA supported the theory of a reaction mechanism including an ionic intermediate rather than a concerted reaction. In the context of doping control analysis, the CIR of DCM, DHEA_S and maybe 5EN17b_S should not be taken into consideration due to the strong isotopic fractionation coming along with the cleavage of the sulfate moiety.