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Determination of $^{13}C/^{12}C$ ratios of endogenous urinary steroids: Method validation, reference population and implication for doping control purposes

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Abstract

The application of a comprehensive gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS)-based method for stable carbon isotopes of endogenous urinary steroids is presented. The key element in sample preparation is the consecutive clean up with high performance liquid chromatography (HPLC) of underivatized and acetylated steroids, which allows the isolation of ten analytes (11β-hydroxy-androsterone, 5α-androst-16-en-3α-ol, pregnanediol, androsterone, etiocholanolone, testosterone, epitestosterone, 5α-androstane-3α,17β-diol, 5β-androstane-3α,17β-diol and dehydroepiandrosterone) from a single urine specimen. These steroids are of particular importance to doping controls as they enable the sensitive and retrospective detection of steroid abuse by athletes.

Depending on the biological background, the determination limit for all steroids ranges from 5 to 10 ng/mL for a 10 mL specimen. The method is validated by means of linear mixing models for each steroid, which covers the items repeatability and reproducibility. Specificity was further demonstrated by gas chromatography/mass spectrometry (GC/MS) for each analyte, and no influence of the sample preparation or the quantity of analyte on carbon isotope ratios was observed. In order to determine naturally occurring $^{13}C/^{12}C$ ratios of all implemented steroids, a reference population of n = 61 subjects was measured to enable the calculation of reference limits for all relevant steroidal Δ values.

A comprehensive publication of the data can be found at:
Piper T, Mareck U, Geyer H, Flenker U, Thevis M, Platen P, Schänzer W: Determination of $^{13}C/^{12}C$ ratios of endogenous urinary steroids: method validation, reference population and application to doping control purposes. Rapid Commun. Mass Spectrom. 2008, 22, 2161-2175