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Measured deviations from its certified values in carbon isotope ratios of the secondary isotopic reference material USADA 33-1 as a function of signal intensity

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Abstract

Within the scope of continuous flow IRMS applications linearity of the mass spectrometer is always crucial as peak heights may vary over a wide range from low mV signals up to more than 10 V. For carbon isotope ratio (CIR) determinations in doping control analysis this might become problematic as here the peak size variability is pronounced due to large concentration differences of all urinary steroids ranging from low ng/mL to midrange µg/mL amounts. Although modern sample preparation methods for gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) were improved to enable separate determinations of most steroids, which allows reconstituting every fraction on its own to reach approximately equal peak height, especially the low concentrated compounds still result in low intensities.

Therefore, the linearity of a GC/C/IRMS apparatus as a whole was systematically investigated by repeated injections at different concentrations of two secondary isotopic reference materials: First the n-alkane mixture C3 containing heptadecane, nonadecane, heneicosane, tricosane and pentacosane purchased from Indiana University and second the USADA 33-1 containing acetylated 3β-hydroxy-5α-androstane (RSTD), 3α-hydroxy-5α-androstan-17-one (ANDRO), 3α-Hydroxy- 5β-androstane-11,17-dione (11KETO) plus underivatized 5α-cholestane (CHOL) from Cornell University.

Four subsets of measurements were conducted with 48 injections of both the n-alkanes and the steroids at 8 different concentration levels respectively summing up in 192 measurements for each class of compounds.

While for the n-alkanes no changes in CIR could be observed over the complete investigated intensity range the steroids showed a non-uniformly response. CHOL and RSTD were not influenced at all but both ANDRO and especially 11KETO exhibited a strong tendency to more enriched CIR with low signal heights. This trend to higher values could partly be compensated for by renewing the connection between the GC column and the combustion tube. Standard deviations increased for both different compounds in parallel with decreasing signal heights as expected. The impact of these findings on doping control analysis will be discussed.

These findings have been published in: T. Piper, C. Emery, A. Thomas, M. Saugy, M. Thevis. Combination of carbon isotope ratio with hydrogen isotope ratio determinations in sport drug testing, *Analytical and Bioanalytical Chemistry*, 2013, 405, 5455-5466.