

“Production of a Certified Reference Material of 19-norandrosterone to support GC/C/IRMS investigation of Adverse Analytical Findings for nandrolone”

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Project Overview

The objective of this project is to further strengthen the ability of anti-doping laboratories to unambiguously identify mis-use of the anabolic steroid nandrolone for athletic performance enhancement. This will be achieved through preparation of a reference material that has an accurately known value for the ratio of stable isotopes of carbon in the chemical residue found in urine after nandrolone is metabolised by the body. The presence in a urine sample of 19- norandrosterone glucuronide, the main urinary metabolite of nandrolone, is indicative of the use of this prohibited drug. Under certain circumstances 19-NA may be present at low concentrations in samples of human urine for reasons unconnected with doping. To confirm Adverse Analytical Findings (AAFs) for samples containing 19-NA at concentrations between 2

ng/mL and 10 ng/mL, WADA Technical document TD2010NA requires that carbon isotope ratio analysis be performed to demonstrate that the metabolite has not been produced naturally in the urine. Provided that other criteria are fulfilled, samples with 19-NA concentrations in this range are only to be reported as an AAF if the carbon isotope ratio of endogenous androsterone in the sample is greater than 3 per mille (‰) different to that of the 19-NA detected.

In this project a stable solution of 19-NA glucuronide with an accurately assigned value for the carbon isotope ratio of the steroid will be prepared. This can then be added by laboratories to a urine CRM free of 19-NA but containing the endogenous reference compound for this analysis (androsterone). The freeze-dried urine CRM MX005, prepared to validate detection of testosterone abuse, has already been assigned a carbon isotope ratio value for androsterone in a previous WADA-funded project and could be used for this purpose.

Results and Conclusions

A certified reference material (CRM) has been prepared in the form of a solution with a metrologically-traceable reference value for the carbon isotope delta value ($\delta^{13}\text{C}_{\text{VPDB}}$) of 19-norandrosterone (19-NA) in its glucuronide conjugate (19-NAG). The CRM has been packaged in 800 amber glass ampoules containing 1 mL of the solution and is now available to WADA-accredited laboratories. It consists of 19-NAG in water containing 20% methanol at a concentration equivalent to 201 ng/mL 19-NA (as the unconjugated steroid).

The certification of the isotope ratio of the 19-NA component of 19-NAG was complicated by the requirement to hydrolyse and remove the glucuronide moiety prior to measuring the isotope ratio of the steroid. The hydrolysis can introduce impurities that must be fully separated from the 19-NA prior to isotope ratio measurement. This was achieved by solvent extraction prior to gas chromatography coupled with combustion isotope ratio mass spectrometry (GC/C/IRMS). Calibration of the GC/C/IRMS was performed using the isotope reference material CU/USADA 34-1, permitting metrological traceability to the international carbon isotope ratio reference standard VPDB. Potential sources of bias in the reference method including isotopic fractionation during extraction, internal standard selection and reproducibility of measurement were investigated. The homogeneity and stability of the CRM was verified by analysis of randomly selected vials after storage at -80 °C, -20 °C and +40 °C for periods up to 10 months.

The certified property value (with uncertainty at the 95% level of confidence) for 19-NA in the CRM is provided in the following table.

	CAS No.	$\delta^{13}\text{C}_{\text{VPDB}} / \text{‰}$
19-norandrosterone	1225-01-0	-29.7 ± 0.8

The CRM is intended for fortification by end users into urine material known to be free of 19-NA in which the $\delta^{13}\text{C}_{\text{VPDB}}$ value for an endogenous reference compound such as androsterone has been well characterised. It is designed to assist laboratories in validation and quality control of methods for determination of the carbon isotope ratio of 19-NA in urine at concentrations in the range 2 – 15 ng/mL as required by WADA technical documents TD2010DL and TD2014NA. The use of the glucuronide conjugate for fortification into urine will permit full validation of the sample workup in analytical methods for confirmation of the presence of exogenous 19-NA in its most abundant metabolised form, 19-NAG.