PROJECT REVIEW

"Certified Internal Standard for accuracy in longitudinal monitoring for testosterone abuse"

Dr. S. Davies, Dr. G. Tarrant (National Measurement Institute, Australia)

The aim of the project is to provide an isotopically labelled internal standard of a key testosterone metabolite. These will facilitate high accuracy measurement of this analyte in human urine thereby contributing to WADAs implementation of the Athletes Passport. The synthesis of d4-epitestosterone-17-b-glucuronide will be performed using a combination of literature precedents and chemistry routinely used by the Chemical Reference Materials team at NMI Australia. This material will be purified to the highest level possible using chromatographic and recrystallisation techniques. The material will then be fully characterised to afford fit for purpose status for use as an internal standard. The identity will be confirmed using a range of spectroscopic techniques including nuclear magnetic resonance, infrared spectroscopy and mass spectrometry. This will be complimented by analysis using a range of traditional techniques (HPLC with ELS detection, Karl Fischer moisture analysis and thermogravimetric analysis) to determine the chemical purity of the material. The isotopic purity will be determined using mass spectrometry on either the deglucuronidated steroid (GC-MS) or direct analysis of the parent material (LC-MS).
Fully certified material will be packaged in 1 mg lots and made available to all WADA accredited laboratories.

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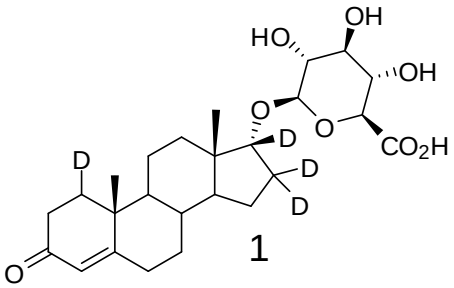
Results and Conclusions:

This summarizes the synthesis of 1,16,16,17-d_4-epitestosterone-17-*O*-β-glucuronic acid (**1)** using a combination of literature precedents and chemistry used previously by the Chemical Reference Materials team. The material has undergone extensive purification to minimise impurities of similar structure.

HPLC analysis with photodiode array detection (PDA) confirmed an organic purity in excess of 99% mass fraction. The overall purity value was lower (~93% mass fraction) than the idealised >99% mass fraction, which can be attributed to significant mass fractions of occluded water. This is typical for glucuronic acids of this type and no attempt was made to remove the water because of concerns that the inevitable re-absorption of moisture would introduce stability issues.

The isotopic purity of each material has been determined using GC-MS analysis of the de-glucuronidated steroid entity, confirming high levels of deuteration, with essentially zero non-deuterated (d0) present, making the isotopically labelled internal standard fit for its intended purpose.



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Chemical Formula: $C_{25}H_{32}D_4O_8$
Molecular Weight: 468.6
Elemental Analysis: C, 64.08; H, 8.60; O, 27.32

The structure of the 1,16,16,17-d_4-epitestosterone-17-*O*-β-glucuronic acid (**1)** has been unequivocally determined using a combination of spectroscopic techniques . Direct comparison with the spectroscopic data obtained for a fully certified sample of non-deuterated epitestosterone-17-*O*-β-glucuronic acid confirmed the regio- and stereochemistry. Further evidence was provided by co-elution studies of the parent compound and/or the liberated steroid moiety, with the fully certified native counterparts.

- Mass spectrometry
- Infra-Red spectroscopy
- ^{1}H / ^{2}H / ^{13}C Nuclear Magnetic Resonance spectroscopy

1,16,16,17-d_4-Epitestosterone-17-*O*-β-glucuronic acid (**1)** was assessed for chemical purity using a complementary range of analytical techniques to determine the mass fraction of the main component. Full characterisation and certification of this material has been performed and each material is currently available to the Australian Sports Drug Testing Laboratory (ASDTL) and other sports doping control laboratories around the world.