

SmartNotes

How do isotope fingerprints support doping control investigations?

QA

While motivated to mark new records with each competition, athletes are more than ever aware of anti-doping policies, rules and regulations. Authorities depend on accurate analysis without exceptions because doping control test results can mean the difference between competing and being banned.

Anti-doping laboratories examine samples such as urine to screen, confirm, and quantify banned drugs. The most common understanding of steroids is that they are drug supplements that enable athletes' enhanced performance. Synthetic copies of endogenous steroids are chemically and pharmacologically identical to their endogenous analogs. Therefore detection of these steroids in urine cannot be used as a proof of administration of performance-enhancing drugs. But analyzing isotopes to provide characteristic **isotope fingerprints** of urinary steroids offers a solution.



To establish this fingerprint, Isotope Ratio Mass Spectrometry (IRMS) is used, measuring the stable isotopes of sample compounds. The application of isotope fingerprints in doping control investigations has become mandatory in doping laboratories worldwide for final confirmation in adverse analytical finding.

Why can isotope fingerprints of steroids be used for doping control?

Variations in the natural abundance of carbon isotopes are defined through biological or chemical processes. As a consequence, isotopic fractionation occurs resulting in slight depletion or enrichment of ¹³C. Pharmaceutically produced anabolic-androgenic steroids are predominantly derived from C3-plant material. Natural isotopic fractionation in C3 -plants results in depletion of the carbon isotope ratios of derived compounds in comparison with endogenously produced steroids.

Regulations for sports doping

The International Olympic Committee (IOC) and the World Anti-Doping Agency (WADA) established gas chromatography coupled with isotope ratio mass spectrometry as a routine analytical technique applied to combat misuse of endogenous anabolic steroids. Doping control laboratories accredited by WADA obligatorily use methods for the determination of the stable carbon isotope ratios (¹³C/¹²C) of steroids in urine.¹

Samples showing suspicious steroid profile parameters are isotopically characterized in order to prove the origin of the steroids. Administration of synthetic steroid copies reveal depleted carbon isotope ratios of the excreted compound and its metabolites. Endogenous reference compounds are not affected and therefore selected for an individual internal standardization of the isotope ratios.

Methodology for carbon and hydrogen isotope fingerprint analysis

The Thermo Scientific™ GC IsoLink II™ IRMS System provides a routine methodology for stable carbon isotope analysis of steroids. After the chromatographic separation of compounds in a GC, the conversion of steroids and their metabolites to a simple gas is performed by combustion of the sample compounds at 1000 °C in the presence of oxygen to evolve carbon from the sample in the form of CO₂. After the gas is produced, it is transferred in helium carrier gas to a detector that measures the carbon isotope fingerprint of the sample compounds. Not only carbon isotope fingerprints can be investigated by GC-IRMS in order to combat drugs abuse, but hydrogen isotope fingerprints (Table 1) might provide some more insights in the future. Pyrolysis, breaking down the sample at 1420 °C in a reductive environment, is used to evolve hydrogen from the sample in the form of H₂ which is subsequently introduced to the isotope detector.

Table 1. Isotope Fingerprints in sports drug investigations.

Isotope Fingerprint	What is the interpretation?	Example forensic interpretation	What sample types can be analyzed?
Carbon	Pharmaceutically produced anabolic-androgenic steroids are derived from C3-plant material which means botanical processes (C3 Photosynthesis) define their carbon isotope ratio value, which finally differs from endogenous steroids.	Distinguishing endogenous anabolic steroids from their synthetic analogs.	Urine
Hydrogen	In anti-doping control it is necessary to know metabolic fate of drugs because knowing metabolites accelerates the drug discovery and method development process.	Deuterium labeling of compounds can be used to identify metabolites in complex matrices.	Urine



No What Ifs - The power of online GC-MS-IRMS coupling

GC-IRMS solely supplies information on isotopic composition of a compound of interest. However, it is possible to couple GC-IRMS with an organic MS system. This simultaneously provides the isotopic compositions and the qualitative and quantitative compound detection, from a single run. Concomitant data are critical to qualify the true identity of a compound.

Analytical solution: detecting isotope fingerprints

Besides doping control, there are dedicated solutions of the Thermo Fisher Scientific™ isotope fingerprinting portfolio designed to offer different capabilities and performances, with dedicated features for the coupling to the Thermo Scientific™ IRMS Systems, according to the varying analytical needs of laboratories working for routine and research applications:

- the Thermo Scientific™ EA IsoLink™ IRMS System, for analysis of bulk sample material;
- the Thermo Scientific™ GC IsoLink II™ IRMS System, for analyzing volatile compounds within a sample;
- the Thermo Scientific™ LC IsoLink™ IRMS System, for analyzing polar compounds within a sample;
- the Thermo Scientific™ GasBench II System, for the analysis of gas samples evolved from bulk sample materials.

References

1. WADA Technical Document, TD2016IRMS.

Find out more at thermofisher.com/IsotopeFingerprints

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