GC-IRMS: Combat emerging threats in drug abuse with isotope fingerprints

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Introduction
Drugs in body are converted in metabolites. These metabolites are removed from body in different ways with greatest portion being excreted through the kidneys. The metabolic fate of drug and the excretion time can vary greatly, causing low detectability of drugs in urine. It is important to study metabolism of therapeutic agents to identify metabolites that provide utmost retrospection and specificity.

Detecting and identifying metabolites in complex matrices is a challenge but necessity. In anti-doping control it is necessary to know metabolic fate of drugs because knowing metabolites accelerates the drug discovery and method development process. Stable isotope labelling is a powerful technique which allows tracking down drug metabolism.

This application brief reports how isotope fingerprints have been effectively investigated to detect labeled metabolites of drug of abuse by using gas chromatography isotope ratio mass spectrometry (IRMS).

Hydrogen isotope fingerprints
Hydrogen isotopes fingerprints are recognized as a valuable mean to identify metabolites. Hydrogen naturally occurs in two stable isotope forms, with $^1$H (Protium) accounting for about 99.98% of all hydrogen atoms that are found on the planet today, and $^2$H (Deuterium) making up the remaining 0.02%.
Urine samples were analyzed by means of the HRMS providing the required sensitivity and mass resolution to further characterize the detected compound. The target metabolite was identified at m/z 304.2731.

Methodology and analytical configuration

Sample injection in the Thermo Scientific™ Trace 1310 GC was performed using a Thermo Scientific™ TriPlus™ RSH autosampler. Compounds eluting from the GC column are in GC IsoLink II preparation unit online converted to a simple gas by pyrolysis. 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. Hydrogen is transferred in helium carrier gas to the Thermo Scientific™ Delta V™ IRMS system that measures the isotope fingerprint of the sample. In addition, GC-IRMS system is coupled with a high resolution mass spectrometer (HRMS). This means that the isotopic compositions and the comprehensive qualitative and quantitative sample information are detected simultaneously.

An elimination study with oral administration of deuterated testosterone has been performed (Figure 1a). 82 urine samples collected before and after the administration were processed according to established sample preparation procedure¹,².

Results

For the steroids detected in the GC-IRMS system during this excretion study (Figure 1.), the amount ranges from 10 to 20 ng/mL for AND and ETIO and falls below 1 ng/mL for T demonstrating the sensitivity of the IRMS approach³.

One new metabolite with unexpected structure (T_METH at 730 s in Figure 1) was detected with the respective mass spectrum not fitting any prediction.