



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

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Goal

Show how the use of isotope fingerprints of labeled metabolites and their corresponding structural information accelerates the discovery of new metabolites.

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 retrospectivity 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 ^1H (Protium) accounting for about 99.98% of all hydrogen atoms that are found on the planet today, and ^2H (Deuterium) making up the remaining 0.02%.

Commonly employed approach to track down drug metabolism is based on deuterium labeling of compounds. A refined methodology which allows for chromatographic separation of compounds in complex matrices, their conversion in H₂ and subsequent hydrogen isotope measurements is provided by Thermo Scientific™ GC IsoLink II™ IRMS System. Small variations of the ²H/¹H can be measured with precision in the range of 1-3‰.

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 procedure^{1,2}.

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.

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.

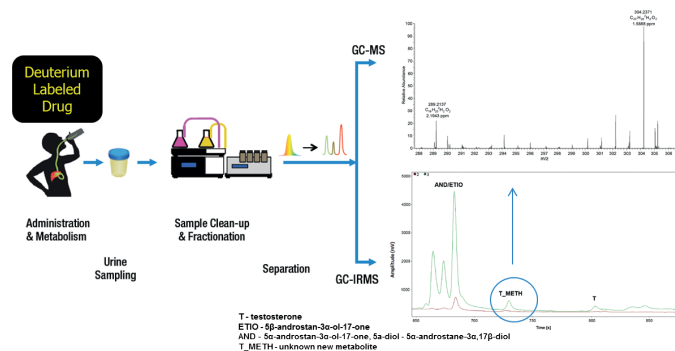


Figure 1. Deuterium stable isotope labeling substantially increases the signal intensities of analytes (green *m/z* 3 trace in GC-IRMS) which lowers the detection limit of drug metabolites. Detected compounds (such as unknown metabolite T_METH) are identified by GC-HR MS.

Summary

Hydrogen isotope fingerprints analyzed by GC-IRMS enable laboratories to stay ahead of metabolites research, exploring new methods and providing solutions to combat drug abuse.

With the GC IsoLink II IRMS System, laboratories gain an effective analytical solution for investigating deuterium labeled metabolites which allows omitting administration of radioactively labeled substances (³H or ¹⁴C) and scintillation counters to detect the compounds of interest. Furthermore, simultaneous identification and structural elucidation of metabolites can contribute and accelerate the drug discovery and development process.

For more details please refer to AN30402

References

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