INSIGHTS ON ISOTOPE RATIO MS FOR FORENSICS

FROM MARINE ACCIDENTS TO MURDERS, THIS TECHNOLOGY HELPS EXPERTS ANALYZE THE EVIDENCE

by Mike May, PhD

Even in chemically identical samples, the ratio of stable isotopes—such as the carbon isotopes ^{12}C and ^{13}C—is not necessarily the same. The isotope ratio depends on the source of the materials and how they were produced. In a biological sample, for instance, the nutrients and the growth environment could change the isotope ratio in the same tissue. As Helen Kreuzer, senior research scientist at Pacific Northwest National Laboratory in Richland, Washington, explains, "A sufficient understanding of how these principles come together in a specific system can allow one to backtrack from stable isotope ratios to synthesis/growth conditions." In some cases, scientists explore these variations with isotope ratio mass spectrometry (IRMS). Dedicated IRMS instruments are used for light isotopes; for heavier isotopes such as lead, other dedicated instrumentation such as inductively coupled plasma MS (ICPMS) is used. The UK-based Forensic Isotope Ratio Mass Spectrometry (FIRMS) Network published an introductory good-practice guide to IRMS, available here: www.forensic-isotopes.org/gpg.html.

Isotope ratios apply to many areas of forensic science. Charles Douthitt, isotope ratio specialist at Thermo Fisher Scientific, headquartered in Waltham, Massachusetts, says, "The measurement of isotope ratios are used in many different types of forensics, including nuclear forensics, wildlife forensics, petroleum forensics, environmental forensics and food forensics. But the two most visible forensics that make use of isotope ratio MS are human forensics and criminal forensics, where the stable isotopes of the 'bioelements'—carbon, nitrogen, sulfur, oxygen and hydrogen—are measured in the materials under investigation."

In general, isotope ratios can be applied to forensics in three ways. First, this measurement could be used to determine whether someone or something is associated with or excluded from a crime. Second, these ratios can reveal the forgery or adulteration of a sample. "Stable isotope ratios have also been used to, for example, detect adulteration of honey with corn syrup," Kreuzer says. Third, the analysis of stable isotopes can indicate where something is from or where someone has been. For instance, isotope ratios in bones can suggest a region where someone lived, while measurement of a drug, such as heroin, can indicate its geographic source.

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As Lesley Chesson, president of IsoForensics in Salt Lake City, Utah, says, "Isotope ratio MS is one of many characterization tools available to the forensic scientist." The chemical basis of isotope ratio variation for light elements comes from fractionation. "The abundance of isotopes in any given sample will vary due to naturally occurring fractionation processes," she says. Across the landscape, for example, the evaporation and condensation of water change the relative abundance of heavy isotopes, like ^{2}H or ^{18}O, versus light isotopes, like ^{1}H or ^{16}O. "As a consequence," Chesson says, "the isotopic composition of water bottled in Texas will be very different—that is, isotopically heavier—than water bottled in Utah."

Kreuzer used stable isotope analysis on a sample of *Bacillus anthracis* spores mailed during the anthrax attacks of 2001. Genome sequences revealed the strain of the bacteria, and the U.S. Federal Bureau of Investigation (FBI) asked Kreuzer whether the mailed spores could have come from a U.S. Army facility in Utah called Dugway Proving Ground. She says, "I had performed enough research delineating the relationships between the isotope ratios of water, nutrients, and spores that I could confidently conclude that the spores had not been produced with water local to Dugway Proving Ground."

She adds, "It's not possible to predict geographic locations from water isotope ratios by zip code—wide regions can have similar isotope ratios—but sometimes you can say with certainty that a particular water source is not consistent with a sample."

▲ *Crimes by mail can be studied with isotope ratio mass spectrometry. (Image courtesy of the U.S. Federal Bureau of Investigation (FBI).)*

Water also plays a part in other forensic samples. In an ongoing investigation of the "Deer Island Jane Doe"—an unidentified child's body found in a plastic bag—hair analysis for water-related isotopes could help. "Because human hair is an isotopic tape recorder, her long hair will retain a record of the last two or three years," Douthitt explains.*

FORENSIC-MATERIAL COMPARISONS

At the Netherlands Forensic Institute (NFI) in The Hague, The Netherlands, experts use IRMS routinely for materials comparison and determining where something is from. For a materials comparison, Gerard van der Peijl, senior forensic scientist at NFI, says, "Typically, a material will be found at a crime scene—for example, gray duct tape used to bind a victim—and on the other hand, a visually similar material will be found with a suspect—for example, a roll of gray duct tape." Using the powerful combination of microscopy, IRMS, laser-ablation ICPMS and other techniques, he says, "We try to determine whether we can discriminate the two materials." Van der Peijl adds, "If the materials cannot be discriminated, there is a link between the materials."

As one might expect, it gets more complicated. "To use the results in court," says van der Peijl, "a further evaluation of the link between the materials is required." For example, the background population variation must be analyzed if not available. "In many investigations, we encounter materials that have not been previously characterized in this manner, and hence the background population variation needs to be established," he explains.

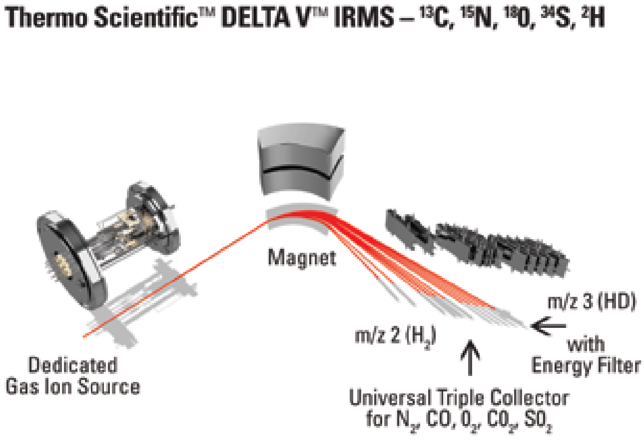
Other studies at NFI involve finding out where an unidentified dead person is from. With the right sample preparation, IRMS—combined with other isotope ratio MS techniques—can reveal unexpected information about someone's background. For example, van der Peijl says, "One of the interesting things in this field is that through analysis of the material of a newborn baby, provenancing information can be obtained [from] the mother of the baby since the baby obtained food only through the mother."

Similar to providing information on where a body may be from, IRMS in combination with other isotope ratio MS techniques can provide information on the provenance of an animal or food.

MORE FROM THE MS

The measurement precision of IRMS is getting better. "For example, there have been recent publications on the measurement of hydrogen and nitrogen isotopes, which are helping to ensure we're more effectively converting samples from solid to gas for analysis, and analyzing all atoms/isotopes of a particular element within a material," Chesson explains. "These methodological developments lead to greater confidence in published data."

As an example of new technology, Douthitt mentions the Thermo Scientific FLASH HT elemental analyzer, which attaches to the Thermo Scientific Delta V IRMS and the Thermo Scientific MAT 253 stable IRMS. He says that it "combines and automates 'Dumas combustion'—for isotopic analysis of carbon, nitrogen, and sulfur—and quantitative carbon reduction—for isotopic analysis of oxygen and hydrogen." He adds, "It can quickly and inexpensively provide large amounts of data that can, in turn, be used with 'isoscapes' to infer plausible geographic location and document movements and diet."



▲*A range of components—including a magnet, filters, and collectors—make isotope ratio mass spectrometry work. (Image courtesy of Thermo Fisher Scientific.)*

More published data also make isotope-ratio analysis even more useful in forensics. In discussing the value of the data, Chesson says, "These datasets—especially when they contain authentic samples of known source—are invaluable for sample comparisons." With these data, scientists build models to predict natural isotope-ratio variation, such as background population variation, in different materials. Nevertheless, "a model is only as good as its foundation," Chesson says, and "better measurement precision and larger datasets lay that foundation."

IT TAKES TRAINING

Only instruction and attention to detail produce reliable results from IRMS. In fact, Chesson says, "Extensive training is required for isotope ratio MS." Beyond using the mass spectrometer, the scientist must prepare the sample properly and use other devices that deliver the sample—as a solid, liquid, or gas—to the mass spectrometer.

Others agree that IRMS is complex. For example, van der Peijl says, "I definitely would not suggest introducing these techniques in a forensic laboratory without previous experience."

The device used for IRMS is sensitive and requires a controlled environment. Even then, other factors must be kept in mind. Chesson explains, "You must monitor analyses for memory effects, area effects caused by varying amounts of sample gas, and instrument 'drift' through time." She adds, "Following standard operating procedures and using reference materials, or standards, regularly are essential for monitoring." A scientist also uses reference materials to normalize data.

ONE TOOL IN THE BOX

On April 20, 2010, the explosion on the Deepwater Horizon oil rig in the Gulf of Mexico triggered a gusher of oil from the Macondo well at the seafloor that continued for 87 days and released about 160 million barrels of oil into the environment. Christopher M. Reddy, a senior scientist in marine chemistry and geochemistry at the Woods Hole Oceanographic Institution in Massachusetts, studied oil from this spill. As he and his colleagues wrote in the Proceedings of the National Academy of Sciences, "Compositional data for released oil is … necessary for forensic analyses when distinguishing Macondo well oil from hydrocarbons released from other sources in the Gulf of Mexico."

In part, these researchers used IRMS. Reddy says, "With environmental forensics, if someone asks me if this oil came from one place or another, I'll build a case around that with a variety of lines of evidence." He adds, "You don't often get a slam dunk with one piece of equipment."

Using a variety of tools, though, Reddy's team showed that the hydrocarbons in the water from this spill differed from those of a sea-surface spill. For this spill, they concluded, "Water-soluble petroleum compounds dissolved into the water column to a much greater extent than is typically observed for surface spills."

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From the seafloor to crime scenes, IRMS can deliver valuable forensic information if the scientist knows the ins and outs of this technology, from sample preparation through data analysis. Furthermore, the technical advances that make this technology more robust help scientists use it in more labs.

* As this issue went to press, authorities solved this murder the old-fashioned way—through an informant's tip.

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