An Executive Summary

Forensic Applications of Isotope Ratio Mass Spectrometry

Lesley A. Chesson

By: Lesley A. Chesson, President, IsoForensics Inc.

Introduction

The isotopic profile or "signature" of a material refers to the ratios of the stable isotopes of elements it contains, such as $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{18}O/^{16}O$. Since biological, chemical and physical processes cause variations in the ratios of stable isotopes, measuring their relative abundance by Isotope Ratio Mass Spectrometry (IRMS) can reveal information about a material's history. Forensic investigators have used IRMS techniques to measure a variety of materials, including illegal drugs, explosives, counterfeit currency, food, beverages, animal tissues like ivory, and human remains. For instance, the isotopes in human hair protein can reveal the date of death of an individual, what s/he ate, and how often and where s/he traveled. This manuscript discusses the application of two common instrument configurations used for gas source IRMS—dual-inlet IRMS (DI-IRMS) and continuous flow IRMS (CF-IRMS)—and how they are useful in the field of forensic science.

IRMS in the News

Many people have read or heard about IRMS in the news without realizing it. In a case from 2006, a popular bicycle race winner was stripped of his title when it was discovered through a urine test that some of the testosterone in his body was not produced by his body at all but was actually from an exogenous, or outside, source. The testosterone source was determined by looking at the carbon isotope ratios in testosterone produced in his body versus synthetic testosterone. Due to isotope ratio differences of the two sources, it was determined that the bicyclist was "doping," resulting in one of the first big court cases in which IRMS was used.

More recently an article was published in *The Economist* (April 2, 2016) about work performed by the US Drug Enforcement Administration (DEA) to test cocaine brought into the country illegally. To determine the original source of the drug, the DEA measured a combination of trace chemical profiles and isotope ratios to form a "telltale 'fingerprint'" of the cocaine.

What Are Isotopes?

Hydrogen, carbon, nitrogen and oxygen are examples of bio-elements found in materials like illegal drugs, human hair, and water. Many bio-elements exist in multiple forms called isotopes, as demonstrated in **Figure 1** for the element carbon. Carbon has six protons in the nucleus, defining its atomic number (6) and place in the periodic table. Most carbon atoms also have six neutrons in the nucleus; the shorthand for this isotope form of carbon is ^{12}C, which is derived from the total number of protons and neutrons. In some rare cases, a carbon atom may contain an additional neutron, giving rise to the ^{13}C isotope, with a different mass: ^{13}C is slightly heavier than ^{12}C because of the additional neutron. The rare ^{13}C form

of carbon is a stable isotope—that is, it doesn't undergo radioactive decay—which behaves a little differently than ^{12}C in biological, chemical and physical processes because of the mass difference. (Another form of carbon has two additional neutrons in the nucleus and is called ^{14}C or radiocarbon; this isotope form of carbon is radioactive and is often used in age dating applications.)

IRMS is used to measure the relative abundance of stable isotopes in a material, or in this example, the ratio of ^{13}C versus ^{12}C. The lighter ^{12}C is the predominant stable isotope form of carbon, accounting for about 98.9% of all carbon atoms that are found on the planet today, with ^{13}C making up the remaining 1.1%. (^{14}C is present in only trace amounts.) A measure of isotopic differences, called "delta" *(δ)* notation, is used to present stable isotope ratios of materials in publications and presentations, as illustrated in **Figure 2**. The $\delta^{13}C$ value, represented in parts per thousand or "per mil" (‰), is determined as the difference of the ratio of the abundances of ^{13}C to ^{12}C in a sample relative to a standard. The standard ensures that isotope ratio measurements made in different labs are on the same scale.

Figure 1: The element carbon exists in multiple isotope forms. Isotopes of carbon have the same number of protons (and electrons)—and thus the same atomic number—but differ in atomic mass due to differences in number of neutrons. An isotope is specified by the total number of protons and neutrons - e.g., ^{12}C, ^{13}C, and ^{14}C. Isotopes can be classified as stable, not undergoing radioactive decay (^{12}C, ^{13}C), or radioactive (^{14}C).

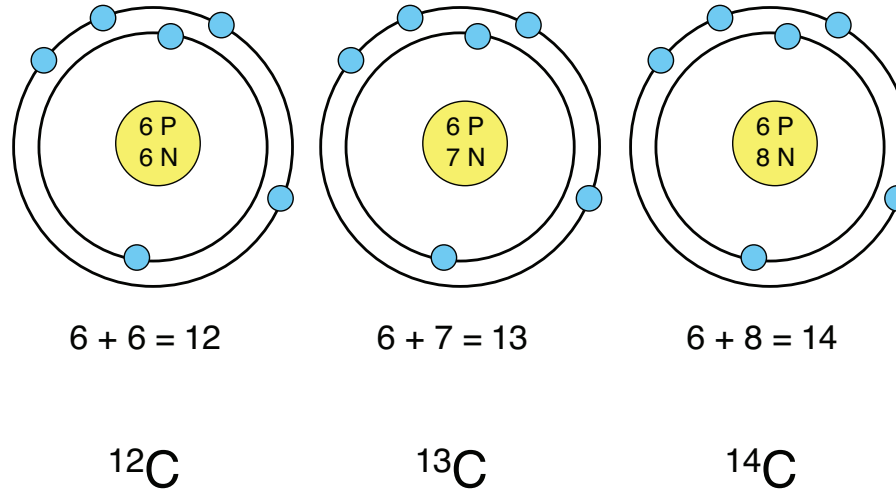


Figure 2: Isotope abundances are measured as ratios and reported using specific notion. This "delta" *(δ)* notation describes the relative deviation of the ratio of two isotopes—in this case, ^{13}C and ^{12}C—for a sample from a standard. Results are typically presented in parts per thousand (‰, "per mil").

$$\delta^{13}C\ (‰) = \left[\frac{^{13}C/^{12}C\ (\text{sample})}{^{13}C/^{12}C\ (\text{standard})}\right] - 1 \times 1000$$

Ensures measurements are on the same scale.

IRMS Instrumentation

There are two common types of IRMS analysis techniques: Dual-Inlet (DI) and Continuous Flow (CF). Both techniques require gas for analysis and so samples in solid or liquid form must be converted to gas for isotope ratio measurement. The DI analysis technique determines isotope ratios from pure gases by alternately introducing sample gas produced offline and a reference gas of known isotopic composition into the mass spectrometer. In CF-IRMS analysis, sample gas preparation occurs inline immediately before gas is introduced to the mass spectrometer and the sample gas is measured just once; reference gas may be measured before or after the sample gas. As the name suggests, CF-IRMS requires the use of a carrier gas. While CF instruments can only measure a sample gas once, as opposed to a DI instrument where sample gas can be measured repeatedly against a reference gas, CF-IRMS is much faster than DI-IRMS because the sample gases don't require an offline preparation. Consequently, most labs today use CF-IRMS in day-to-day operation, but will often have a DI setup to use when high precision is required.

CF-IRMS systems consist of five major components:

- A sample introduction system
- An electron ionization source
- A magnetic sector analyzer
- A Faraday-collector detector array
- A computer-controlled data acquisition system

A basic CF-IRMS instrument schematic is shown in **Figure 3**. The most common sample introduction systems are elemental analyzers (EA-IRMS; **Figure 4**) and gas chromatographs (GC-IRMS; **Figure 5**); liquid chromatographs (LC-IRMS) have also been used in limited applications. By performing a separation of sample components prior to gas preparation, techniques such as GC-IRMS and LC-IRMS can provide isotopic analysis of individual compounds isolated from a complex mixture, thereby providing additional characterization information for samples.

The basic role of the sample introduction system is to convert the sample to simple gases—such as CO_2 for the measurement of carbon isotope ratios and N_2 for the measurement of nitrogen isotope ratios—which are then separated via gas chromatography (GC). The electron ionization source ionizes these resultant gases, which are separated by the magnetic sector analyzer on the basis of their mass to charge ratio (m/z). Faraday cups collect the ions, and the resultant signal is processed into a measured isotope ratio. Returning to the carbon example – there are three masses of interest in the measurement of carbon isotope ratios, for CO_2 molecules containing the stable isotopes of ^{12}C, ^{13}C, ^{16}O, ^{17}O, and ^{18}O in various combinations:

- $^{12}C^{16}O^{16}O$ . . . . . mass 44
- $^{13}C^{16}O^{16}O$ or $^{12}C^{17}O^{16}O$ . . . . mass 45
- $^{12}C^{16}O^{18}O$ . . . . . mass 46

Elemental Analysis Isotope Ratio Mass Spectrometry (EA-IRMS)

There are two main forms of elemen-

Figures 3-5: Schematics representing major components of isotope ratio mass spectrometry (IRMS) systems. Continuous flow (CF) instruments have five distinct sections: a sample introduction system, an electron ionization source, a magnetic sector analyzer, a Faraday-collector detector array, and a computer-controlled data acquisition system. Two common sample introduction systems are the elemental analyzer (EA-IRMS, **Figure 4**) and gas chromatograph (GC-IRMS, **Figure 5**).

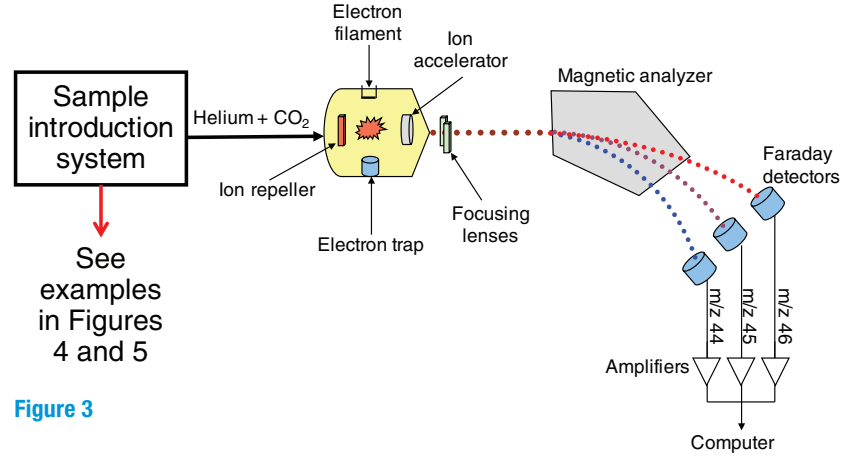


Figure 3

Muccio and Jackson (2009) *Analyst* 134: 213–222

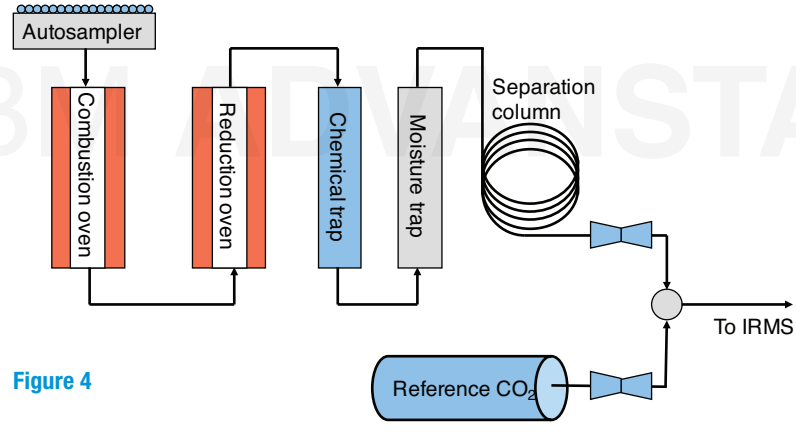


Figure 4

Muccio and Jackson (2009) Analyst 134: 213–222

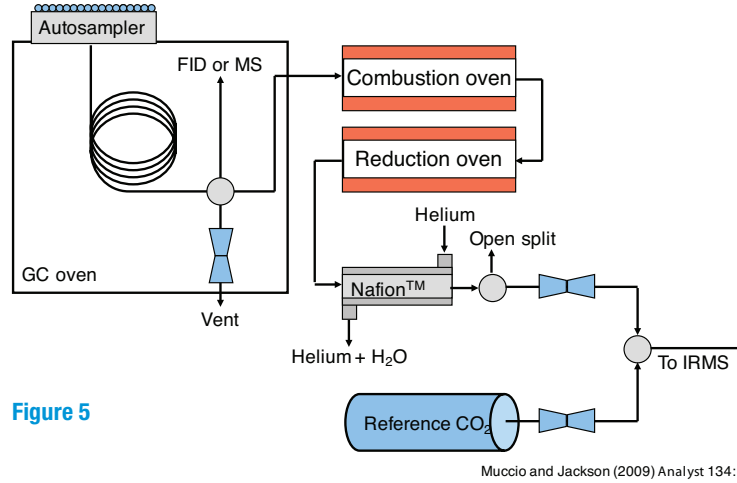


Figure 5

Muccio and Jackson (2009) Analyst 134: 213–222

tal analysis used in IRMS: one in which the samples are combusted (EA) and another in which the samples go through a high temperature thermal conversion (TC/EA). Basic EA and TC/EA systems consist of an autosampler, one or two reactors, and traps to remove impurities like water before the sample gases are separated for isotopic analysis.

In EA systems (**Figure 4**), a sample is dropped into a reactor and oxygen is introduced, causing the tin capsule, in which the sample is enclosed, to combust. Evolved gases are carried through a column of catalyst, typically chromium oxide, to ensure complete oxidation and then through a column of chemicals, typically silver cobaltous oxide, to remove halide and sulfur compounds. Next, the combustion gases pass through another oven containing reduced copper to remove excess oxygen and to convert nitrogen oxides to N_2. The final stage is water removal. At this point, the carrier gas stream should only contain N_2 and CO_2 gases, which are separated using GC.

TC/EA systems are generally much simpler than EA systems. These systems are comprised of an autosampler and a single reactor, partially packed with glassy carbon providing a strong reducing environment in which the hydrogen and oxygen present in water or organic materials are converted to H_2 and CO. Samples are introduced to the oven enclosed in silver capsules. A plug of silver wool in the reactor serves to remove halide and sulfur compounds. The H_2 and CO gases evolved from samples are then separated using GC.

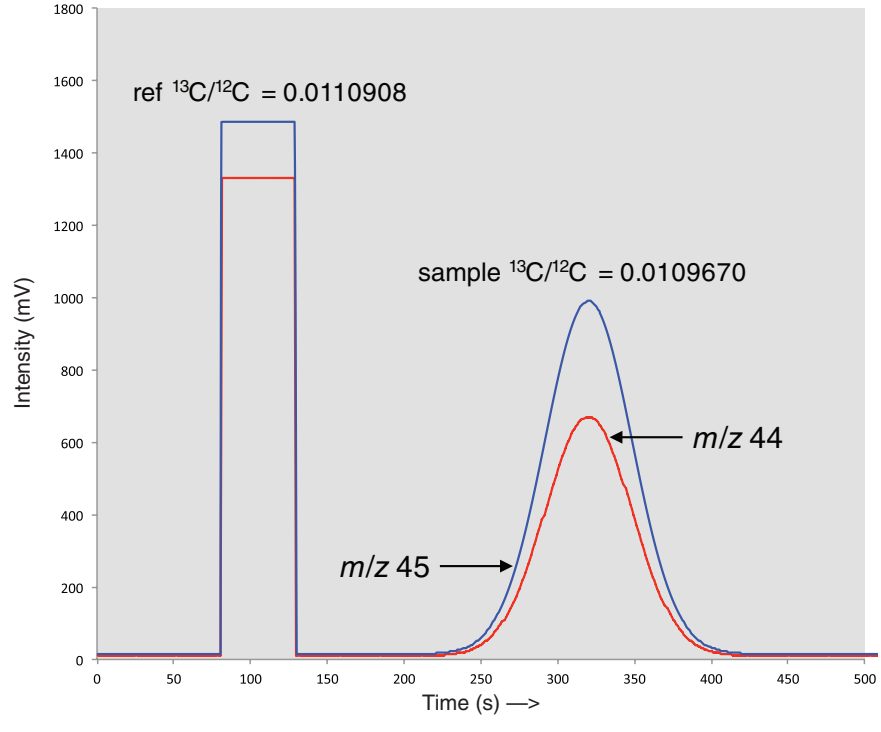
IRMS Data Analysis

Isotope ratio mass spectrometers are very good at determining relative differences between sample and reference gases. Because the isotope ratios for questioned samples are reported via the computer-controlled data acquisition system relative to a reference gas, best results are obtained when the signal intensities for the two peaks are of similar magnitude and are measured as closely together in time as possible. **Figure 6** shows a typical response from monitoring the ions of CO_2. The traces of the signals for *m/z* 44 and 45 are shown in red and blue respectively. The sample gas response is the Gaussian-shaped peak on the right; the reference gas response is the square-shaped peak on the left. If the exact carbon isotope ratio of the reference gas is known, a δ-value for the sample can be calculated by comparing the ^{13}C to ^{12}C ratios determined from the sample and reference peaks.

To place the δ-value in context, and to compare data collected by different labs, calibration through the use of reference materials must be done to express the sample's isotopic composition on an international isotope scale. Primary (calibration) materials define the δ-scales versus which natural variations in isotope ratios are expressed; by definition, these materials have a δ-value of 0‰. However, many of the materials no longer exist. Secondary (reference) materials are commercially available natural or synthetic materials that have been carefully calibrated to the primary reference materials and have an isotope ratio determined by collaboration between expert laboratories. These reference materials are quite expensive and usually available only in very small quantities and on a limited basis to prevent their exhaustion.

For day-to-day operation, laboratories will use secondary reference materials to calibrate in-house standards, which are then directly traceable back to the original primary calibration materials. Using these laboratory standards in analysis ensures results are comparable between different labs, as the known difference between δ-values of two known standards ($\delta_{std1} - \delta_{std2}$) is a constant value between labs. Normalization, a specific correction technique using these laboratory standards, is necessary to compare results from day-to-day, instrument-to-instrument, or laboratory-to-laboratory analyses

Figure 6: CF-IRMS instruments continuously monitor ionic species of interest using multiple Faraday cups. Here, signals for CO_2 (*m/z* 44 and 45) are shown for the reference gas (square peaks) and sample gas (Gaussian peaks).



by correcting for any drift, shift, or nonlinearity in instrument output. For these reasons, laboratory standards ***must*** be included in every analysis.

Examples of IRMS Used in Forensic Settings

Examples of the application of IRMS include food production, evidence comparisons, and origin determinations. Isotopes of the same element have the same number and configuration of protons and electrons and therefore have virtually the same chemical properties. However isotopes differ in mass and consequently can behave differently in biological, chemical and physical processes. The process by which the isotope ratio of a material is changed through partitioning of isotopes is called fractionation. For example, the abundance of isotopes in a leaf is affected by fractionation processes. When fixing CO_2 from the atmosphere into starch, plants preferentially use $^{12}CO_2$ versus $^{13}CO_2$ and the strength of that preference is different in plants using different photosynthetic pathways. There are two major photosynthetic pathways: C_3, in which CO_2 is first incorporated into a 3-carbon molecule and C_4, in which CO_2 is first incorporated into a 4-carbon molecule. These different pathways result in distinctly different δ-values for C_3 and C_4 plants. Most fruits come from C_3 plants, while common sweeteners (sugar cane, high fructose corn syrup) are derived from C_4 plants. Armed with this information, investigators can, for example, determine how much fruit is really in a particular jam, what sugar source is being used to produce a certain beer, or whether C_4 sugar has been added to sparkling fruit wine to encourage additional fermentation.

Fractionation processes also affect the hydrogen and oxygen isotopes of water. Ocean water contains both heavy and light isotopes, and as ocean water evaporates, the resulting water vapor contains fewer heavy isotopes (i.e., is isotopically lighter) than the ocean. That water vapor moves inland and cools, forming a cloud. As precipitation falls from the cloud, water that is isotopically heavier than the cloud, yet isotopically lighter than the ocean, is removed and the water vapor left behind in the cloud is isotopically lighter still. Fractionation in the water cycle creates a systematic, predictable pattern of meteoric water isotope ratios across land surfaces. These patterns can be displayed in an isotope landscape or "isoscape." Although the isotope ratios are not "zip code" specific, they can be used to distinguish geographic regions and can help to to determine the origin of animals and plants, as the isotope ratio signals are incorporated from water into biological tissues such as hair and cellulose.

In 2000, IRMS was used to assist in the investigation of an unidentified set of human remains that were found by the Great Salt Lake in Utah. By looking for differences in the oxygen isotope ratios in hair segments, it was possible to predict where the individual had traveled based on changes in her drinking water isotopes. These predictions ruled out some potential locations and highlighted others for additional investigation. The analysis eventually led to the discovery of a missing person's report that fit many features of the remains, and a DNA test between living relatives confirmed the identity of the unknown remains.

Conclusion

Materials that are chemically indistinguishable by other analytical techniques can be distinguished by stable isotope ratio analysis. Stable isotope analysis by IRMS provides an additional signature to the chemical signature of a sample and generates characterizing information for use in forensic investigations.