Isotope ratio mass spectrometry

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Isotope Ratio Mass Spectrometry (IRMS) is a specialized technique used to provide information about the geographic, chemical, and biological origins of substances. The ability to determine the source of an organic substance stems from the relative isotopic abundances of the elements which comprise the material. Because the isotope ratios of elements such as carbon, hydrogen, oxygen, sulfur, and nitrogen can become locally enriched or depleted through a variety of kinetic and thermodynamic factors, measurement of the isotope ratios can be used to differentiate between samples which otherwise share identical chemical compositions. Several sample introduction methods are now available for commercial isotope ratio mass spectrometers. Combustion is most commonly used for bulk isotopic analysis, whereas gas and liquid chromatography are predominately used for the real-time isotopic analysis of specific compounds within a mixture. Here, highlights of advances in instrumentation and applications within the last three years are provided to illustrate the impact of this rapidly growing area of research. Some prominent new applications include authenticating organic food produce, ascertaining whether or not African elephants are guilty of night-time raids on farmers’ crops, and linking forensic drug and soil samples from a crime scene to a suspected point of origin. For the sake of brevity, we focus this Minireview on the isotope ratio measurements of lighter-elements common to organic sources; we do not cover the equally important field of inorganic isotope ratio mass spectrometry.

Introduction

Isotope ratio mass spectrometry (IRMS) is a technique which finds increasingly widespread use in disciplines such as archaeology, medicine, geology, biology, food authenticity, and forensic science. The histogram plot in Fig. 1 shows the number of publications per year containing the research topic ‘Isotope ratio mass spectrometry’ using SciFinder Scholar 2006 (searched on May 3, 2008) and reflects the rapid growth in applications since the introduction of commercially-available instrumentation approximately ten years ago. The fastest growth is arguably in forensic applications, where the ability to differentiate substances by their geographical origins provides information that is difficult or unattainable by any other technique.

Disciplines which stand to benefit from IRMS are those which require the ability to accurately and precisely measure variations in the abundance of isotopic ratios of light elements such as $^{12}\text{C}$/$^{13}\text{C}$, $^{16}\text{O}$/$^{18}\text{O}$, D/$^{2}$H, $^{15}\text{N}$/$^{14}\text{N}$, and $^{34}\text{S}$/$^{32}\text{S}$. The ratios of these isotopes are always measured relative to an isotopic standard in order to eliminate any bias or systematic error in the measurements. These standards are, or can be linked to, internationally recognized standards such as Vienna Pee Dee Belemnite (VPDB).

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Valkiers secondary standards must sometimes be used in their place. Primary standards can become environmentally depleted, and example, halogenated hydrocarbons are reported between 0.76 and 27 \( \text{mg L}^{-1} \). Detection limits vary according to the analyte: for monoaromatic compounds between 0.07 and 0.35 \( \text{mg L}^{-1} \), and are reported in the delta notation, \( \delta \). The value \( R_{\text{standard}} \) is the abundance ratio of the minor, heavier isotope of the element to the major, lighter isotope (e.g. \( ^{13}\text{C}/^{12}\text{C} \)). Samples which establish the \( R_{\text{standard}} \) values are usually selected because they represent a stable material which is highly enriched in the heavy (minor) isotopes. Most analyzed substances are depleted in the heavy-isotope relative to the standard and will therefore have negative delta values. Guidelines for the selection of working standards and a review of strategies to institute universal isotopic referencing procedures have been reported by Werner and Brand.\(^6\)

Commonly used mass spectrometers such as single quadrupoles, ion traps, and time-of-flight mass spectrometers typically do not provide the sensitivity or precision required to detect the subtle differences in naturally-occurring isotopic abundances. It should be noted that these instruments can be useful when used with isotope dilution\(^7\) – a technique in which the heavier isotopes are deliberately enriched well beyond their natural levels. However, the measurement of natural isotopic abundances requires a specialized instrument such as a multi-collector magnetic sector mass spectrometer, also known as an isotope ratio mass spectrometer (IRMS). Several authors have investigated the precision and accuracy of IRMS. Continuous flow IRMS instruments have shown precisions of 0.1\%\(_{\text{oo}}\) with the lowest reported detection limits for monoaromatic compounds between 0.07 and 0.35 \( \text{mg L}^{-1} \). In general, detection limits vary according to the analyte: for example, halogenated hydrocarbons are reported between 0.76 and 27 \( \text{mg L}^{-1} \), which is significantly higher than the limits seen for monoaromatics. Although the analyte is also the most important variable in instrumental performance, certain benchmarks in accuracy and precision can be reasonably anticipated. Wong et al.\(^8\) tested three commercially-available GC-IRMS instruments to determine differences in precision and accuracy. The average precision was 0.12\%\(_{\text{oo}}\), with reproducibility of 1.48\%\(_{\text{oo}}\) and accuracy of \(-1.11 \pm 2.16\%\). Additional experimental variables such as the stability of the ion current,\(^7\) dead time, bit board size dependencies,\(^8\) and even the possibilities of sample vial influences\(^9\) can all effect precision and accuracy on individual instruments.

Another technique which can be used for isotope ratio measurements is known as multiple collector inductively coupled plasma mass spectrometry (MC-ICP-MS). MC-ICP-MS is a technique which has undergone extensive research to enhance the accuracy and precision of stable isotopic measurement.\(^10\)–\(^13\) Clough et al.\(^4\) have demonstrated that MC-ICP-MS can be used as a high-throughput tool for the \( \delta^{34}\text{S} \) measurements of bulk aqueous and solid samples, using Si as an internal standard for correction of instrumental mass bias effects in both pure solutions and in samples with high matrix content. This technique is limited, by plasma instabilities and the performance of data acquisition in sequential mode, to the identification of large variations in isotopic abundances.

There are five main sections of an IRMS instrument: a sample introduction system, an electron ionization source, a magnetic sector analyzer, a Faraday-collector detector array, and a computer-controlled data acquisition system. Several different interfaces are used to introduce samples into the IRMS, the two most common being elemental analyzers (EA-IRMS) and gas chromatographs (GC-IRMS). Fig. 2 demonstrates how each of these sample introduction systems can be coupled to the same mass spectrometer. Although liquid chromatographs (LC-IRMS) have recently gained interest for some applications, there are only a limited number of publications that have shown this technique to be successful. Here, we examine the present state of research involving IRMS and explore some of the most interesting and unusual applications.

**EA-IRMS**

EA-IRMS is a bulk measurement technique which provides representative data for the average isotopic signal of the entire sample. Without significant sample preparation, this method
cannot divulge how each constituent of the sample contributes to the total average value. In order to measure the average isotope ratios for non-volatile liquids or solids, the bulk sample can simply be weighed and placed in a tin or silver capsule. The sample capsule is lowered into a combustion furnace through an autosampler carousel, at which time the sample is combusted at elevated temperatures under a flow of oxygen into NO\textsubscript{x}, CO\textsubscript{2}, SO\textsubscript{2}, or H\textsubscript{2}O. Depending on the isotopes of interest, the combustion products may need to be specifically treated to reduce interferences. In carbon isotope ratio analysis – by far the most common application – the combusted sample is carried by a helium gas stream into a reduction chamber where nitrous oxides are converted into N\textsubscript{2} and excess O\textsubscript{2} is removed. The analyte is next carried through a chemical trap to remove water that was produced from combustion, and then into the gas chromatograph where separation of CO\textsubscript{2} and N\textsubscript{2} is performed. Effluent from the elemental analyzer is then sent to the IRMS. Because the isotope ratios for questioned samples are reported relative to a reference gas standard, best results are obtained when the signal intensities for the two samples are of similar magnitude and are analyzed as closely together in time as possible. The flow of each reference gas is regulated using a dedicated sample introduction interface system which toggles quickly between the reference gas and the sample using pneumatic actuators.

**GC-IRMS**

By performing a separation prior to isotope ratio analysis, hyphenated techniques such as GC-IRMS and LC-IRMS can provide isotopic analysis of a complex mixture, thereby providing additional information and higher discriminatory power. IRMS instruments require a somewhat steady stream of a fixed gas (such as CO\textsubscript{2}) for precise analysis. The sample first elutes from the GC column into an oxidation chamber, usually housed on the side of the GC oven. The oxidation chamber is normally a non-porous alumina tube that contains three separate twisted wires made of copper, nickel, and platinum. The samples are combusted at elevated temperatures into a combination of gases such as CO\textsubscript{2}, NO\textsubscript{x}, and H\textsubscript{2}O. For δ\textsuperscript{13}C measurements, the combusted sample is then carried into a reduction chamber where nitrous oxides are converted into N\textsubscript{2} and any excess O\textsubscript{2} is removed. Since CO\textsubscript{2}, NO\textsubscript{x}, and H\textsubscript{2}O will not condense at room temperature, the transfer line from the oxidation chamber to the reduction chamber does not need to be heated. The reduction chamber and subsequent valves, splitters, and pneumatic actuators, etc., are contained in a stand-alone interface system. To avoid H\textsubscript{2}O from protonating CO\textsubscript{2} in the MS source – and causing deleterious isobaric interference of \textsuperscript{12}CO\textsubscript{2}H\textsuperscript{+} with the \textsuperscript{13}CO\textsubscript{2}\textsuperscript{+} peak at \textit{m/z} 45 – the analyte stream is passed through a semi-permeable membrane such as Nafion™. Here, a dry helium...
counter-flow is used to remove the H₂O. The flow rate of the subsequent sample stream is carefully controlled to provide a stable flow rate to the IRMS ion source of approximately 0.5 mL min⁻¹. Deactivated fused silica capillaries are used throughout the interface system to restrict the analyte flow to the required flow rates. The interface system also uses electronically-controlled pneumatic actuators to toggle the flow of the effluent stream between that of the analyte and that of a reference gas, such as a cylinder of CO₂.

LC-IRMS

LC-IRMS applications are typically dedicated to carbon isotope ratio analyses. When the solution elutes from a high pressure liquid chromatograph (HPLC) column, the solution is directly injected onto or into one of two interfaces. These two interfaces are (1) a moving wire interface, and (2) a wet-chemical oxidation interface.¹⁵ The wet-chemical oxidation interface is currently being offered as a commercial instrument by Thermo (LC IsoLink) and shows somewhat more promise¹⁶–²¹ than the moving wire interface, which only has one prototype. The wet-chemical oxidation method converts organic compounds present in the effluent of the HPLC column into CO₂ gas directly in the mobile phase. To reduce interferences, the HPLC mobile phase must be void of any organic or oxidizable components that could interfere with the results. It should be noted that because most HPLC separations are greatly enhanced with organic solvents or modifiers, this requirement poses significant restrictions on the potential application of LC-IRMS. The effluent from the HPLC column is then mixed with a stream of an oxidizing agent such as ammonium peroxodisulfate, and a catalyst such as phosphoric acid and silver nitrate.¹⁷ The mobile phase and the combined reagents pass through a capillary oxidation reactor where the organic compounds are converted into CO₂. A membrane exchanger separates CO₂ gases from the other gases (water vapor, oxygen, argon, etc.) that originate from the liquid phase. The CO₂ is then transferred through a gas-permeable membrane into a counter-flow of helium. The CO₂ in the helium stream is then dried in an online gas drying semi-permeable membrane (Nafion™) and admitted to the isotope ratio mass spectrometer via an open split. The wet-chemical oxidation interface allows for the ¹³C/¹²C determination of organic compounds with a completely automated online high precision method.

Origins of variations in isotopic abundances

Although the average isotope ratio of each terrestrial element was fixed around the time of the earth’s formation, localized variations occur based on selective enrichment/depletion of the heavier isotopes relative to the average values. For example, even though all plants use atmospheric or dissolved CO₂ as a source of carbon, various factors can influence a plant’s ability to enrich or deplete ¹³C from these common sources in a process known as fractionation. One such fractionation factor is genetic. Monocotyledonous plants (C₄ plants), such as sugar cane, corn, tropical grasses, desert plants and marine plants, utilize the Hatch–Slack photosynthetic cycle.²¹ These plants typically have δ¹³C values varying from −8 to −20‰.²³ Most dicotyledons plants (C₃ plants), such as flowering plants, wheat, rice, rye and cotton employ the Calvin–Benson photosynthetic cycle and have δ¹³C values varying from −22 to −35‰.²³ Crassulacean acid metabolism (CAM) plants, such as pineapple, cactus, and orchids, can utilize either the C₃ or C₄ metabolic systems, depending on sunlight, and therefore have δ¹³C values ranging between −10 and −34‰.²³ Because animals can only incorporate carbon through the ingestion of plant or animal matter, the carbon isotope ratios in an animal will reflect the isotope ratios of the food source; i.e. ‘you are what you eat’. This fact can be used to great advantage, as shown in Fig. 3. For example, human European diets are richer in C₄ plants (wheat, barley, and rye), whereas human North American diets are richer in C₃ plants (corn, sugar cane and millet). Therefore, a person living in North America will have body matter with isotope ratios more similar to C₄ plants and will have lower ¹³C levels (i.e. less negative δ values) relative to Europeans.

In addition to genetic factors, environmental factors such as temperature, rainfall, and hours of sunlight also influence fractionation. These factors can influence kinetic processes such as the diffusion of CO₂ through the stomata in plant leaves. Clear evidence for environmental sensitivity to fractionation was presented by Ehleringer et al. in 2000, wherein they demonstrated the ability to determine the local geographic farming regions in South America from which different cocaine plants were obtained.²⁴

Fractionation also occurs in common elements such as sulfur, hydrogen, oxygen and nitrogen. In the case of sulfur, fractionation occurs in an equilibrium (between reactants and products) and non-equilibrium (kinetic) mode. Kinetic effects are due to fast, incomplete, or unidirectional processes, typically resulting in a preferential enrichment of the lighter isotope in the reaction products.²⁵ Grassineau²⁶ studied fractionation of both carbon and sulfur and concluded that it is possible to limit the effects of fractionation with careful attention to detail. Hydrogen fractionation was studied by Maruoka et al.,²⁷ who showed that hydrogen comparatively has the most extreme fractionation

![Image](https://example.com/image.png)
effects. Bond strength also plays an important role in kinetic effects due to the greater strength of a deuterium–carbon bond relative to a hydrogen–carbon bond.\textsuperscript{28} Oxygen fractionation is largely due to the combustion of the sample, with temperature a deciding factor as to whether or not the sample is completely combusted. If the sample is only partially combusted, or if the oxygen levels are depleted in the oxidation chamber, this can affect the results of the isotopic ratio. It has also been shown that oxygen fractionation can occur within a sample vial.\textsuperscript{19} Additionally, oceanic vapors have had a large effect on the oxygen content.\textsuperscript{29} Nitrogen fractionations in nature are due to kinetic effects: there are also two non-biological fractionation effects, dissolution in water and diffusion in water. Bacteria, in particular, display several fractionation processes: nitrification, denitrification, and nitrogen fixation.\textsuperscript{29}

General fractionation also occurs with ambient diffusion.\textsuperscript{29} Chemical reactions and physical processes like evaporation and condensation create products that are isotopically distinct from their starting materials.\textsuperscript{30} For example, in the hydrologic cycle, snow falling at the poles is depleted in \({\text{^2}H}\) (D) and \({\text{^18}O}\) content with respect to rainfall at the equator.\textsuperscript{30} Fractionation effects are also observed in purely chemical reactions. As a result, any simple or complex substance will be composed of isotope ratios that provide a key in unravelling the history and origins of its precursor elements. This fact has been pivotal in solving a variety of interesting and important problems, as described below. For additional information, a complete description of isotope fractionation effects for \({\text{^13}C/\text{^12}C, \text{^18}O/\text{^16}O, \text{D}/\text{H, ^15}N/\text{^14}N, ^34}S/\text{^32}S, \text{and several others not mentioned in this Minireview, was written by Mook and Vries.}\textsuperscript{29}

### Applications

#### Forensic

Forensic science researchers have long-recognized the need to distinguish between different sources of evidential material. Such determinations were formerly difficult or impossible in cases where two samples had identical physical or chemical properties. However, isotopic analysis now provides a means to look beyond the chemical composition of matter to the level of the nucleus. To help monitor and disseminate the developing forensic applications of isotope ratio mass spectrometry, a specialized network called the Forensic Isotope Ratio Mass Spectrometry (FIRMS) Network was developed in January 2002. This voluntary network is mostly composed of British and European members from universities and forensic laboratories. FIRMS have also organized several inter-laboratory studies as well as the European Institute for Reference Materials and Measurements (IRMM) and the National Physical Laboratory (NPL). An extensive review of analytical chemistry inter-laboratory studies was written by Hund \textit{et al.},\textsuperscript{31} while an excellent example of an inter-comparison study was that done on tetramethyleurea by Breas \textit{et al.}.\textsuperscript{32}

Until recently, the notion that the carbon isotopic ratio of soil could be used to determine whether soil recovered from a suspect matched a crime scene would have been regarded as implausible. The use of carbon and nitrogen EA-IRMS analysis of soil to partially exonerate a suspect is thus yet another example of how far the forensic applications of IRMS have advanced.\textsuperscript{33} In another application, it was proven that a carbonate rock was switched during transit from a supplier’s factory in South Africa to a client’s factory in Israel.\textsuperscript{34} Isotopic analysis of the carbon in the sample indicated that the origin of the carbonate rock was likely the client’s site in Israel, which substantiated the claim that the rock was switched after arrival. Further elemental flexibility of EA-IRMS was exhibited by the analysis of carbon, oxygen, and hydrogen in matches.\textsuperscript{35} The results of these tests indicated that matches recovered from a suspect’s house and crime scene were different, which underscores the potential importance of this technique to criminal justice. EA-IRMS has also been used to measure the carbon isotopic abundance of 28 samples of white architectural paint.\textsuperscript{36} Specifically, the effects of drying time, layering, ageing, and homogeneity on discriminatory results were reported.

As a result of constant legal demand, the analysis of controlled drugs is one of the largest areas of active development. For example, the presence of elevated levels of \(\gamma\)-hydroxybutyric acid (GHB) in blood, urine, or hair samples is often necessary to support claims of drug-facilitated sexual assault (DFSA). GC-IRMS of carbon isotopic ratios has been used to discriminate between endogenous production of GHB and exogenous ingestion levels.\textsuperscript{37} Fig. 4 shows the IRMS results of such an analysis. The figure shows the \textit{m/z} 44 peak for the esterified version of GHB (\(\gamma\)-butyrolactone; GBL) and the internal standard \(\epsilon\)-caprolactone. In a broader sense, IRMS may also be useful in the struggle against drug trafficking. A notable illustration of this application is the nitrogen isotopic comparison of 20 samples of heroin seized from a North Korean-flagged cargo vessel \textit{versus} a database of more than 200 authentic samples. It was determined that the samples from the seizure had not originated from a source currently on record and were therefore likely to have come from a new source.\textsuperscript{38}

With the threat of terrorism omnipresent in our modern lives, techniques are needed to address unique problems associated with mass-disasters. For example, the ability to reconstruct a victim’s remains following an event of mass destruction is useful from both a forensic point of view and to provide closure for the families and friends of deceased victims. When DNA cannot be collected, or when DNA reference samples are not available, IRMS is a potential option to link recovered body parts to each other or to a geographic location. EA-IRMS has been used to analyze the hydrogen and oxygen isotope ratios of human scalp hair and fingernails.\textsuperscript{39} In another case,\textsuperscript{40} isotope ratio analysis of oxygen and hydrogen from an unknown deceased person in Ireland was used to verify that the person almost certainly had not lived in the local geographical region for a significant amount of time. Isotopic analysis instead suggested that the person was likely from Eastern Europe or Scandinavia. Further studies by the same authors proved that hydrogen isotope ratios provided much more valuable information when analyzing human hair than when analyzing nails.\textsuperscript{41} In a separate study, a model was developed to predict the geographical region of North America from which a person resides by cross-referencing the hydrogen and oxygen isotopic abundances of scalp hair with tap water.\textsuperscript{42} For the initial data in this model, individual hair samples were collected from 65 cities across the United States, while tap water samples were collected from 18 states. The
accuracy of this model was confirmed by collecting hair samples from local barbershops in the same cities and comparing them with previous samples. The findings showed an agreement of 85% with the model, which relies upon the assumption that all hair in the local barbershops originated from indigenous citizens. A more-thorough review on forensic applications of IRMS, including earlier studies of explosives and synthetic drugs, was written by Benson et al.

Food and drugs

In the food and drug industries, it is necessary to determine whether or not a product’s actual contents agree with the labeled contents. Although food and drinks are sometimes laced with relatively innocuous substances such as artificial sweeteners, they are not always marked as such. Isotope ratio analysis can be used to establish whether or not the product contains natural sweeteners (from the original food source), or artificial sweeteners (such as corn syrup). A recent study showed that sugars could be analyzed using LC-IRMS to obtain carbon isotopic ratio abundances of each sugar of interest.20 Because of their similar carbohydrate contents, cheap beet sugars are sometimes undetected when added to honey products as sweeteners. However, through the isotopic analysis of each sugar within the questioned honey samples, it is possible to determine which honeys are altered and which are not. Moreover, the detection of other modifiers such as corn, sugar cane, or sweeteners other than high fructose corn syrup is possible. In a similar vein, an internal standard of malic acid, found only in very low abundances in commercial sweeteners, was employed to study which of 56 selected maple syrup samples were unsweetened.21

Other food products known to be altered are fruit juices and sparkling drinks. Authentic sparkling drinks are usually pressurized with CO₂ through a fermentation process, whereas cheaper and easier method for carbonation is to pressurize the drinks with CO₂ from an external cylinder. GC-IRMS can detect the different modes of carbonation by testing the carbon and hydrogen isotope ratios of natural versus injected CO₂.43 In a different application to food produce, IRMS was used for the quality assessment of apple aroma profiles in apple juices.44 Analysis of food additives, in this case citric acid, for the authentication of fruit juices were also studied using 20 commercial citric acids and 79 citric acids extracted from fruit juices.45 A disadvantage encountered in this study was that the exchangeable hydrogen sites bound to the oxygen atoms are included in the overall D/H result. Therefore, an offline preparation step was necessary. The capability of the developed method to detect an addition of citric acid was confirmed by spiking an orange juice sample with known amounts of citric acid.

The increasing popularity of organic produce has been accompanied by prices that are significantly higher than non-organic produce. To validate whether or not more expensive produce is truly organic, and thus warrants the higher prices, isotope ratio analyses have been used to authenticate organic produce. Based on the premise that ‘you are what you eat’, the isotope ratios of flesh (meat) from animals such as cows are determined by their feed source. Bahar et al.46 investigated the seasonal variations of beef using EA-IRMS to study the carbon, nitrogen, and sulfur isotopic ratios of 242 beef samples. Between the months of December and June an isotopic shift was apparent, most likely due to indoor winter feeding practices. By applying the shift during these months, it was possible to use isotopic ratios to determine whether or not a beef sample was indeed organic or merely conventional Irish beef. In a related study, the accuracy of beef-rearing labeling was questioned in part by measuring the hydrogen and oxygen isotopic ratios of lipid fractions with EA-IRMS.47 Carbon and nitrogen isotope composition of beef defatted dry mass comprised the other component of the analysis. The study suggested that it was not
only possible to determine where the beef was reared, but also to validate the accuracy of the information on the labels.

Although the above analyses require that the animal be sacrificed prior to analysis, it is also possible to test for isotope ratios without these invasive measures. For example, a non-invasive method to test carbon and nitrogen isotopic abundances has been performed using urine and milk from cattle. Specifically, isotope ratio analysis helped determine which type of feed was being used during what season. Further, from the identity of the feed, it was possible to distinguish whether or not the beef production was organic or conventional.

**Diet, biochemistry and metabolism**

Isotopic abundance ratios can also establish dietary patterns and movements of cattle. One such study reconstructed the dietary history of cattle by measuring the carbon and nitrogen isotopic abundances of bovine hooves with EA-IRMS. Specifically, the keratin within the hoof was used in order to establish the short-term dietary changes and history of the cattle. This was taken a step further using a three-dimensional growth of the bovine hoof to study the seasonal and ontogenetic feeding patterns, as well as the movement, of the cattle. Feeding patterns have also been characterized using bone collagen to perform palaeodietary reconstruction and distinction between marine- and C₄-based diets. To make this contrast, LC-IRMS was used to separate 18 amino acids from modern protein and archeological bone collagen hydrolysates taken from human and faunal bone collagen. Bone collagen has also provided evidence that indicated maize as the primary source of sustenance in many regions of the Central Andes during the era of Inka hegemony. The effects of preferential fertilization of maize relative to manure were determined by using the carbon and nitrogen isotopic ratios within the bone collagen. To augment these findings, muscle and skin from mummies dating back to the late prehistoric early colonial (AD1490–1640) time from Peru’s Ayacucho Valley was analyzed as well.

When dealing with non-volatile compounds such as amino acids or fatty acids, extensive sample preparation or derivatization is needed prior to introduction into the GC-IRMS. This adds complexity to the data interpretation, particularly if the number or type of atoms in the molecule is increased through the derivatization process. Corr et al. demonstrated a less extensive derivatization method using four novel derivatives on 15 amino acids. N-Acetyl methyl esters (NACMEs) were shown to add the fewest amount of carbon atoms and resulted in the smallest δ¹³C errors relative to the underivatized amino acids. The same authors later confirmed these findings by utilizing this derivatization technique to study amino acids from rat tissue (bone collagen).

Zoology has also been the beneficiary of IRMS research, such as in the study of the dietary and migration patterns of elephants by Cerling et al. This project used an EA-IRMS analyzer to determine the carbon and nitrogen isotopic ratios of hair taken from wild elephants, as shown in Fig. 5. Segmented analysis of the hair from the elephants was then used to generate a chronological history of the elephants’ eating habits, and even their feeding locations. The isotope ratios for the average of all the elephants in the study showed gradual changes from season to season. The results also showed that an individual elephant’s hair could be significantly different from the control group if the elephant in question was involved in night-time crop raiding. Such differences are only possible when the crops under discussion are of a different metabolic class than the control group’s native diet.

**Fig. 5** Carbon and nitrogen isotope ratio analysis of wild elephants’ hair can be used to detect the occurrence of night-time raids on farmers’ crops. (Adapted with permission from ref. 55. Copyright 2006, National Academy of Sciences.)
Another zoological study examined the migration patterns of wild birds. Using feathers from vertebrate and invertebrate species of birds, the migrating patterns as a function of isotopic carbon and hydrogen abundance ratios were developed. Additional analysis of the birds’ summer plower feathers established the bird’s origin within several kilometers of their known origin. An earlier, related report of migratory patterns of other species can be found in an article by Bowen et al. on global applications of stable hydrogen and oxygen isotopes in wildlife forensics. In short, isotope grids were used to statistically constrain the unknown origin of North American and European feathers and water isotopes.

Medical problems such as digestion patterns and mechanisms have also been an area of interest. A recent study examined carbohydrate digestion and glucose absorption by measuring plasma glucose with EA-IRMS after oral administration of naturally-occurring $^{13}$C-enriched carbohydrates. By using Saccharomyces cerevisiae (yeast) to convert the samples into $^{12}$CO$_2$, the magnitude of glucose digestion was determined. Another biochemical investigation used a tracer from blood to measure the fractional synthesis rate of glutathione (GSH) after infusion of (1-13C)-glycine. Amino acids from low birth weight infants admitted into the neonatal intensive care unit were analyzed using LC-IRMS. To achieve higher resolution, the oxidated form of glutathione, GSSR, was used: this tracer made it possible to determine both the individual carbon isotopic ratio of GSSR and the fractional synthesis rate of glutathione.

Given current circumstances, environmental investigations may be among the most important IRMS applications. Environmental effects are known to contribute to carbon isotopic abundance ratios of plant matter, although the manner and extent of this interaction is still a mystery. To partially address this question, the effects of turbulent water on the CO$_2$ flux of the boundary layer of CO$_2$ on the plant surface, which in turn causes faster diffusion rates and thus noticeable differences in the isotopic ratios of fixed CO$_2$. In this application, EA-IRMS was used to analyze the levels of carbon isotope ratio variability of the torrenticolous families Podostemaceae and Hydrostachyaceae.

**Athletics and doping**

Doping in the athletics world has been a problem for decades. Many sports governing bodies rely on isotope ratio analyses to determine elevated levels of exogenous sources of illicit steroids versus elevated levels of endogenous hormones, which is possibly indicative of a genetic anomaly. Because of its specificity, IRMS is the preferred analytical technique to confirm steroid use. GC-IRMS has been used to analyze urine for the carbon isotope ratios of nandrolone (NAD), an endogenous steroid hormone metabolite used to enhance the performance of race horses and athletes. One of the most abundant metabolites of the synthetic steroid 19-nortestosterone is 19-norandrosterone. Utilizing reference compounds to compare isotopic carbon ratios to distinguish between endogenous and exogenous concentration levels, EA-IRMS determined the origin of urinary norandrosterone traces. Urine samples can also be a vehicle to detect steroid use. To detect the effects of undecanoate over a four-week period, GC-IRMS was used to measure the carbon isotope ratios of androsterone and etiocholanolone metabolites in seven Caucasian male volunteers.

**Environmental pollution**

For obvious public health reasons, the effects of pollutants on the environment are an area of great research importance. In a common post-industrial scenario, sediment with elevated polycyclic aromatic hydrocarbons (PAHs) found in a lakebed near a former gas-manufacturing plant was studied. Carbon isotopic analysis of the sediment samples was carried out using a GC-IRMS to prove that the samples were not the same as the tarry soil samples recovered from the gas plant. Instead, results indicated that the hydrocarbons most likely came from a mixture of PAH sources such as coal tars and carbureted water gas tars. Perhaps the most significant contemporary environmental issue is that of global warming. Keppler et al. found that it was possible to use lignin methoxyl groups within wood to determine past climatic changes. Lignin methoxyl is a major component of wood (up to 3%) contained in cellulose cell walls and is produced by secondary metabolic processes. The researchers discovered that converting lignin methoxyl into CH$_3$I made determination of hydrogen isotopic ratios via EA-IRMS straightforward. In the future, it is hoped that this method could reconstruct annual climate histories and assist in ecophysiological research.

**Summary**

IRMS has been shown to have both wide applicability and versatility to be coupled with several different interfaces. In determining which interface would be best suited for coupling to the IRMS, the sample itself is the most important determining factor. Non-volatile substances such as foods, drugs, amino acids, and fatty acids can be most easily measured with EA-IRMS, even though this technique only provides an average isotope ratio value for the entire sample. Analysis can typically be performed on samples as small as 0.5 mg and often avoids the complex sample preparation procedures that are usually needed for GC- or LC-IRMS analysis. With that said, it is important to note that GC-IRMS can be used for most volatile organic substances without sample preparation. LC-IRMS is still the least mature sample introduction method. As such, it seems that the most important obstacle in this technique is ensuring adequate baseline-resolution, in the absence of organic modifiers, in the chromatographic stage of the analysis. Regardless of the sample introduction method, IRMS has great potential for forensic applications where large amounts of illicit drugs are dealt with, such as burglary, homicide, and drug dealing crimes. In the future, the analysis of drugs using IRMS will undoubtedly become more common for both controlled and illegal drugs. A particularly key benefit is the possibility of linking trace amounts of drugs to a bulk source in order to determine trafficking routes. A noticeable gap in the market exists for compound-specific isotope ratio standards, although several suppliers are available for bulk isotope ratio standards such as polyethylene, sugar, and flour. Thus, the availability of IRMS standards and standardized methods are both important goals. Another major current and
future issue is the rendering of compatible isotopic measurement referencing strategies. To address this issue, Serra et al. have already begun development of a standardization method for inter-laboratory δ¹³C elemental analysis and gas chromatography combustion isotope ratio mass spectrometry measurements. Several suitable compounds for GC-IRMS isotopic reference materials were investigated in order to comprise a standardized Grob-test. For forensic applications, validated sampling protocols and sampling kits would be additionally advantageous.

Improvements continue to be made as researchers find new ways to utilize this technique. IRMS offers the potential of unlimited applications for non-volatile and volatile compounds while achieving higher accuracy and precision via increased automation. Through continued progress in fundamental understanding and application development, IRMS should be able to transition from its current status as a specialized practice into a more routine method. Future instrumentation goals could then focus on shrinking the footprint and cost of the instrumentation, reducing analysis times, obtaining higher resolution data, and perhaps even looking towards miniaturization or portable instruments.

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