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Forensic Science Applications of Stable Isotope Ratio Analysis

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Stable isotope ratio analyses have been commonplace in the environmental, biological, and geological fields for many decades [1, 2]. The use of stable isotopes in forensic studies is relatively new, but is now rapidly expanding because of the many ways this analytical approach can help with law enforcement investigations [3]. Stable isotope analyses can complement and link with other analytical approaches to chemical identification in an investigation (e.g., HPLC, GC/MS, LC/MS), because stable isotope analyses provide an additional "fingerprint" that further characterizes a piece of forensic evidence. Stable isotope

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analyses provide a means of relating or distinguishing two pieces of evidence that have exactly the same chemical composition (e.g., TNT explosive material found at a crime scene and at the suspect's residence). The study of stable isotopes as a forensic tool is based on the ability of an instrument to precisely measure very small, naturally occurring differences in the amounts of the heavy stable isotopes in evidence material and to relate that composition to other samples or other evidence. That evidence can be in the form of specific compounds (e.g., TNT), mixtures (e.g., heroin), tissues (e.g., bird feathers, hair), or other materials (e.g., packaging tape, food items). In order to conduct a stable isotope measurement, a special type of mass spectrometer called an isotope ratio mass spectrometers is used to separate the light from heavy isotopes of an element. In this chapter, we will focus on the applications of gas isotope ratio mass spectrometers, which are routinely used to analyze the stable isotope ratios of hydrogen (H), carbon (C), nitrogen (N), oxygen (O), chlorine (Cl), and/or sulfur (S) in evidentiary materials.

15.1 WHAT ARE STABLE ISOTOPES?

Elements are identified on the basis of the number of protons in their nucleus. Atoms of an element share a common number of protons; however, they may differ in the number of neutrons contained inside the nucleus. Different forms of an element are based on the numbers of neutrons within the nucleus—each form is called an isotope. Stable isotopes are those isotopes of an element that are stable: that is, they do not decay through radioactive processes over time. Most elements consist of more than one stable isotope. For instance, the element carbon (C) exists as two stable isotopes, ^{12}C and ^{13}C, while the element hydrogen (H) exists as two stable isotopes, ^{1}H and ^{2}H (also known as deuterium). Table 15.1 provides the average stable isotope abundances of those elements applicable to forensic investigations.

TABLE 15.1 Abundances of Stable Isotopes of Light Elements Typically Measured with an Isotope Ratio Mass Spectrometer

Element	Isotope	Abundance (%)
Hydrogen	^{1}H	99.985
	^{2}H	0.015
Carbon	^{12}C	98.89
	^{13}C	1.11
Nitrogen	^{14}N	99.63
	^{15}N	0.37
Oxygen	^{16}O	99.759
	^{17}O	0.037
	^{18}O	0.204
Sulfur	^{32}S	95.00
	^{33}S	0.76
	^{34}S	4.22
	^{36}S	0.014

Stable isotopes should not be confused with radioactive isotopes of an element, such as ^{14}C (also referred to as radioactive carbon) or ^{3}H (also called tritium). Radioactive isotopes have limited lifetimes and undergo a decay to form a different element, although the time required for this decay may vary widely ranging from nanoseconds to many thousands of years. For instance, carbon has five very short-lived radioactive isotopes (^{9}C, ^{10}C, ^{11}C, ^{15}C, and ^{16}C) with lifetimes of seconds to minutes and one longer-lived radioactive isotope (^{14}C) with a half-life of 5710 years. Radioactive ^{14}C is perhaps best known because of its utility in dating biological materials that are less than 50,000 years old and as a tracer in metabolic studies.

15.2 WHAT ARE THE UNITS FOR EXPRESSING THE ABUNDANCE OF STABLE ISOTOPES?

Stable isotope contents are expressed in "delta" notation as δ values in parts per thousand (‰), where $\delta‰ = (R_s/R_{Std} - 1) \times 1000‰$, and R_s and R_{std} are the ratios of the heavy to light isotope (e.g., $^{13}C/^{12}C$) in the sample and the standard. We denote the stable isotope ratios of hydrogen, carbon, nitrogen, and oxygen as $\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$, respectively.

R values have been carefully measured for internationally recognized standards. The standard used for both H and O is Standard Mean Ocean Water (SMOW), where $(^{2}H/^{1}H)_{std}$ is 0.0001558 and $^{18}O/^{16}O$ is 0.0020052. The original SMOW standard is no longer available and has been replaced by a new International Atomic Energy Agency (IAEA) standard, V-SMOW. The international carbon standard is PDB, where $(^{13}C/^{12}C)_{std}$ is 0.0112372 and is based on a belemnite from the Pee Dee Formation. As with SMOW, the original PDB standard is no longer available, but IAEA provides V-PDB with a similar R value. Atmospheric nitrogen is the internationally recognized standard with an R value of $(^{15}N/^{14}N)_{std}$ of 0.0036765. Lastly, the internationally recognized standard for sulfur is CDT, the Canyon Diablo Troilite, with a value of $(^{34}S/^{32}S)_{std}$ of 0.0450045. Typically, during most stable isotope analyses, investigators would not use IAEA standards on a routine basis. Instead, laboratories establish secondary standards to use each day that are traceable to IAEA standards and that bracket the range of isotope ratio values anticipated for the samples.

15.3 WHAT IS THE BASIS FOR VARIATIONS IN STABLE ISOTOPE ABUNDANCES?

The abundances of heavy and light stable isotopes of an element vary in nature because of both physical and biological processes. Isotopic enrichment is defined as the difference in the isotope ratio of a reactant (R_r) and a product (R_p) as $\alpha = R_r/R_p$. An approximation in delta notation that is often used is $\varepsilon = (\alpha - 1) \times 1000$, where $\delta_p = \delta_r - \varepsilon$. Three specific processes tend to affect a

distribution in the abundances of stable isotope concentrations, resulting in an isotope effect (α) between substrate and product. These are equilibrium fractionation events, kinetic fractionation events, and diffusion fractionation events. Equilibrium fractionation events reflect a difference in the stable isotope ratio of a compound where there is reversible movement of the molecule between two phases, such as between water as vapor and water as a liquid. In such cases, there is a clear tendency for the heavier stable isotopes to remain in the lower energy form (liquid). Kinetic fractionation events are considered as irreversible steps, such as enzymatic reactions between a substrate and product. Again, heavier sable isotopes are more thermodynamically stable, and thus less reactive. Consequently, there is a tendency of the heavier isotopes to react less, resulting in the product being isotopically lighter than the substrate. Lastly, in the gas phase, there is a diffusion fractionation whereby molecules with heavier stable isotopes tend to diffuse more slowly than molecules with light stable isotopes.

15.4 WHAT INSTRUMENTATION IS NEEDED FOR HIGH-PRECISION STABLE ISOTOPE MEASUREMENTS?

High-precision measurements of the stable isotope abundance in a known compound or material are made by converting that substance into a gas and introducing the gas into a mass spectrometer for analysis (Figure 15.1). The

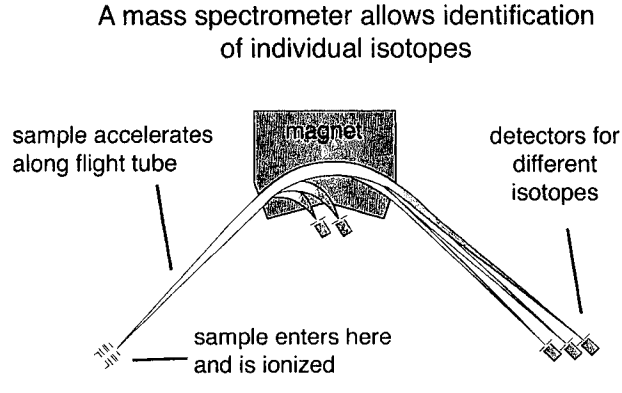

Figure 15.1 *Diagram of a stable isotope ratioing mass spectrometer. Prior to entering the mass spectrometer, the sample is typically completely combusted and the resulting gas is introduced into a gas chromatography, system, where the gases produced during combustion are separated. The pure gas sample (e.g., CO_2 or H_2) then enters the ionizing chamber, is accelerated along the flight tube where it encounters a magnetic field from a fixed magnet, and is deflected into mass-specific Faraday cups. Offline gas preparations can also be introduced to the mass spectrometer.*

instrument for the measurement of the stable isotopes of H, C, N, O, Cl, and S is referred to as an isotope ratioing mass spectrometer (IRMS). Typically, the elements of interest are introduced as the following gases: H as H_2, C as CO_2, N as N_2, O as CO or CO_2, Cl as CH_3Cl, and S as SO_2. At the inlet to the mass spectrometer, the purified gas is ionized and the ion beam is then focused and accelerated down a flight tube, where the path of the ion species is deflected by a magnet. Based on the different isotopic compositions of the ions, they are differentially deflected by the magnet. For instance, for measurements of the carbon isotope composition of a material, the carbon is oxidized to produce CO_2 and the primary species formed are $^{12}C^{16}O^{16}O$ (mass 44), $^{13}C^{16}O^{16}O$ (mass 45), and $^{12}C^{18}O^{16}O$ (mass 46). In contrast to a traditional mass spectrometer, where the strength of the magnet is varied and the ionic species are measured by a single detector, in the IRMS the magnetic field is fixed and the different isotopic ionic species (three in this example) are deflected into separate detector cups at the end of the flight tube, allowing for greater sensitivity in the measurement of the ratio of $^{13}C/^{12}C$. While a traditional mass spectrometer may be able to detect a 0.5% difference in the R_s value of $^{13}C/^{12}C$ in a sample such as might occur in ^{13}C-enriched biochemical studies, an IRMS has the capacity to resolve a 0.0002‰ difference in the R_s value at the low end of the naturally occurring $^{13}C/^{12}C$ range. In the case of CO_2, this is because R_s is measured in an IRMS as the ratio of the simultaneous currents in the two cups: $^{12}C^{16}O^{16}O$ (mass 44) and $^{13}C^{16}O^{16}O$ (mass 45) detectors.

An elemental analyzer and/or gas chromatograph are often coupled to the front end of the IRMS. With such an arrangement, it is then possible to oxidize the sample for C, N, or S isotope analyses using the elemental analyzer, to separate the different combustion gases using a gas chromatograph, and to analyze the sample gases as they pass into the inlet of the IRMS (so-called continuous-flow IRMS). In this case, helium is used as a carrier gas, transporting the combustion gas products from the elemental analyzer to the mass spectrometer. Similarly, it is possible to pyrolyze the sample in the elemental analyzer for hydrogen and oxygen isotope ratio analyses. In addition, samples can also be isolated, purified, and combusted offline and then introduced into the mass spectrometer for stable isotope analyses.

In a stable isotope analysis, a standard of known isotopic composition is analyzed before or after the sample is analyzed. This improves the accuracy of a measurement by directly comparing the isotope signals of the sample and the working standard with each other. As a consequence, a daily calibration is not used with the instrument, because essentially every sample is compared to a standard treated to the same preparation and analysis conditions. These working standards are identified by the stable isotope community and are exchanged among different laboratories in order to determine the best estimate of the actual stable isotope composition of the working standard. Under the best of conditions, there will be many working standards, reflecting a range of stable isotope ratios and a range of material types (e.g., water, plant material, animal tissue, explosive).

15.5 HOW CAN STABLE ISOTOPE ANALYSES ASSIST FORENSICS CASES?

Stable isotope ratio analyses yield potentially unique information for forensic investigations, complementing other approaches by adding a "stable isotope signature" to compounds or materials that are identified as identical by other methods (see Figure 15.2). Key applications of stable isotope ratios include sample matching, sample processing information, and source location identification. Several samples of a material of interest (e.g., illicit drugs, counterfeit money, toxins) may be obtained by investigators with the intent of identifying related groups or sources of the material [4]. Biological samples obtained from the same location and time will have experienced the same environmental conditions, they will therefore have similar stable isotope ratios [5]. Chemical processing of materials can also result in distinctive stable isotope ratios, as can impurities left behind during the production of derived products [6, 7]. Because of this, like samples may readily be grouped based on their stable isotope ratio signatures.

In addition to matching samples, forensic workers can compare individual samples to databases of stable isotope ratio information obtained from

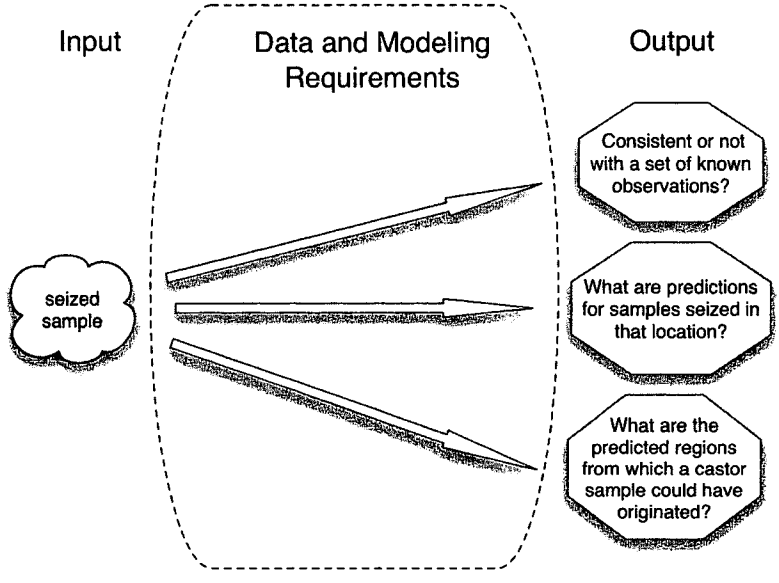

Figure 15.2 *Stable isotope ratio analysis of forensic samples. Three primary classes of information can be provided for seized samples based on stable isotope ratio data. Samples can be matched either to other samples seized or to samples previously obtained and recorded in a database. If the database is sufficiently extensive or if other modeling has been completed (see text), then two additional classes of questions can be addressed: What are the expected stable isotope ratios of samples from a given location and given a sample stable isotope ratio, from where might that sample have originated?*

authentic samples [8]. In this case, a sample of unknown origin could be assigned probable source location by comparison with an authentic stable isotope ratio database, or a previously made assignment of origin could be corroborated by database comparison. A novel approach to geographical assignment may yet yield even greater information, especially in the case that an incomplete database is available. By using first-principles models of stable isotope fractionation in organisms and the spatial modeling capacity of Geographic Information Systems, spatial maps of predicted stable isotope ratios can be constructed [9]. A sample stable isotope ratio can then be compared to the map predictions, and potential source locations can be identified. These advances represent cutting edge applications of stable isotope ratios to forensics that will continue to develop over the coming years. As databases and fundamental understandings of stable isotope ratios grow, so will their capacity to aid in forensic work.

15.6 STABLE ISOTOPE ABUNDANCES IN FORENSIC EVIDENCE

Natural isotope fractionation processes and fractionation steps associated with the synthesis of different products often lead to a wide range of stable isotope ratio values. Consider three kinds of biological samples that might be analyzed in a forensic case: human hair, drugs, and food. There is often a wide range of values in these materials, allowing for an opportunity to detect stable isotope ratio differences among samples. For example, the carbon isotope ratios of human hair can range from –25‰ to –10‰, depending on the human's diet. On the other hand, carbon isotopes in food can range from –30‰ to –10‰, depending on the plant's photosynthetic pathway. However, if plants are grown indoors with a carbon dioxide supply (such as indoor grown tomatoes), the carbon isotope ratios can be as low as –50‰. In drugs, the biologically derived drugs often have carbon isotope ratio values that overlap with the carbon isotope values of foods, but when synthetic drugs are included, the range of values can be much greater and is dependent on the starting materials that are used for the synthesis. It is this variation of stable isotope ratios in forensic, biological, and commercial samples that allows stable isotope analyses to add additional useful information in characterizing a sample or set of samples under consideration in a forensic case. A significant concern here is within-sample heterogeneity. This variability can make it difficult to distinguish among samples that have similar stable isotope ratios. It is normally the case that the precision of any single measurement from the mass spectrometer is more precise than that of the entire sample because of heterogeneity. This heterogeneity is often greater in biological samples than synthetic samples, because of small differences in the chemical make-up of different biological tissues. An understanding of the expected heterogeneity in a set of samples is therefore necessary prior to the interpretation of any stable isotope ratio data.

15.6.1 Food Products, Food Authenticity, and Adulteration

Plants can be divided into two photosynthetic pathway groups: C_3 and C_4 photosynthesis [10]. These two pathways exhibit distinctive differences in their carbon isotope ratio values, with C_3 plants tending to have carbon isotope ratios of about –27‰ and C_4 plants having a value of about –12‰ [11]. Among the foods we eat, most tend to be C_3 plants, including most grains, fruits, and starchy foods. In contrast, the most common C_4 plants are warm-season grasses, which include corn, sorghum, millet, and sugarcane.

Products of high commercial value are often adulterated to increase profits through substitution using a low-cost alternative. A common example of adulteration would be the dilution of honey (a product ultimately derived from C_3 plants) by the addition of low-cost fructose corn syrup (a C_4 plant product). Even though the corn-sugar substitution cannot be detected chemically through traditional GC/MS or HPLC analyses of a honey sample, IRMS analyses can distinguish between real and adulterated honeys by carbon isotope analyses [12]. Here isotope ratio analyses have also proved quite useful in detecting adulteration of imports, such reconstituted fruit juices, where water has been added back to concentrate [13] and artificial vanilla sold as true vanilla from vanilla beans [14].

Adulteration of food products can easily be observed in common U.S. foods. Consider two commonly consumed alcoholic beverages: beer and sparkling wines. Beer should be brewed with barley or wheat grain, hops, and water. Each of the biological ingredients is from C_3 plants and therefore we might expect the carbon isotope ratio of beer to be about –27‰. However, it is clear from an examination of the carbon isotope ratios of beer that many of the domestic beers have carbon isotope ratios that are consistent with a 50:50 C_3:C_4 mixture (Figure 15.3). In Europe, where there are authenticity laws, the carbon isotopes of beer tend to be C_3-like, consistent with barley or wheat as the primary ingredient. In contrast, the U.S. NAFTA partners appear to be producing both C_3 beers (expected) and C_3/C_4 mixture beers (unexpected). As a second example of beverage adulteration, wines should be produced from grapes, another C_3 plant, and therefore the carbon isotope ratios should be approximately –27‰. To their surprise, Martinelli et al. [15] observed that many of the sparkling wines from the United States, Brazil, and Australia were mixes of a C_3 component (grapes) and a C_4 component (most likely corn or sugarcane fructose). This was particularly true for wines from Australia, Brazil, and the Untied States. In contrast, sparkling wines from Argentina, Chile, and France tended to exhibit only C_3 signals. While the legality of these practices might be questionable, the absence of ingredient labeling requirements for these food products certainly opens the door for incorporation of corn or sugarcane sugar as a mechanism to boost profits. Where adulteration of a food product has been taken seriously has been in cases of U.S. product protection from foreign sources. For example, U.S. ATF regulations on the carbon isotope ratios of acceptable honeys has resulted in a shift in

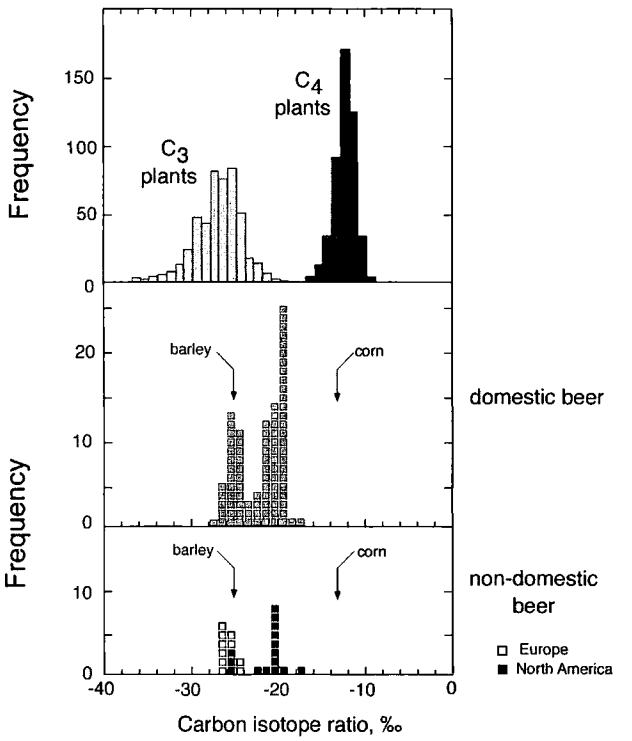

Figure 15.3 *Plants with C_3 versus C_4 photosynthetic pathways have distinct, nonoverlapping carbon isotope ratio distributions. Since barley is a C_3 plant and corn is C_4, the detection of corn addition to beer is readily made. In this example, the European beer had a strong C_3 stable isotope ratio signature, whereas the North American beer showed clear addition of corn, with an average calculated proportion of 50% corn, 50% barley.*

carbon isotope ratios of the received samples away from C_4 adulteration values [12].

Concerns over illnesses such as mad cow disease, testing compliance with food production regulations, and verification of claims of origin, especially for high-value products such as cheeses or wines, are all contributing to an increased use of stable isotope analysis in food authenticity [16, 17] (see also http://trace.eu.org). One of the early applications of isotopes to food authenticity was the identification of origin and adulteration in honey using carbon isotope ratios [18]. Carbon isotope ratios of plants that use the C_3 photosynthetic pathway differ significantly from plants that use the C_4 pathway. Adulteration of honey with C_4 sugars is therefore easily detected with carbon isotope analysis. Carbon isotope ratios have also been applied to maple syrups, other fruit juices, jams, vanillin, olive oil, and other sugars as a method of detecting adulteration [19, 20]. In addition to the detection of sugar additions, other applications exist such as for the detection of synthetic steroids in meat production with the same protocols for detection of human doping [21].

In addition to $\delta^{13}C$, both $\delta^{18}O$ and $\delta^{2}H$ have been utilized extensively for food authentication. Since, in addition to internal biochemical transformations, climate and geography affect plant $\delta^{18}O$ and $\delta^{2}H$ values, these have been used to authenticate both food sources and processing [17]. As an example, wine oxygen and hydrogen stable isotope ratios have been quite extensively explored as useful markers of geographic source, production methods, and even vintage [22–26], although the current methods require large databases of authentic samples for comparison with suspect samples. The combination of multiple isotopes can yield a rich array of information on food production methods and geographic sources. For example, although the existing research is currently fairly limited, the combination of hydrogen, oxygen, nitrogen, and sulfur isotopes has been explored for the detection of methods of farming beef, as well as its geographic source [27]. Combining light isotope ratio analysis with heavy isotope ratios (e.g., lead or strontium [28–30]), or with other chemical or elemental analyses [31], also promises to yield rich information about food sources and processing methods.

15.6.2 Doping and Drugs of Abuse

Illicit drugs have been shown to have stable isotope ratio values that characterize the region from which a drug sample originated. An "isotopic fingerprint" has been detected in marijuana, heroin, and cocaine, usually as a combination of the carbon and nitrogen isotope ratio values. Earlier studies were constrained by a limited number of authentic exhibits but clearly established the comparative value of isotope analyses in comparing different heroin samples [32–34]. Ehleringer et al. [35] showed that the carbon and nitrogen isotope ratios of heroin from the major poppy growing regions of the world clustered into four distinct groupings, allowing for the identification of the region of origin of different heroin exhibits.

However, it should be noted that heroin is a synthetic molecule, as it is the product of acetylation of morphine with acetic anhydride. As a consequence, any isotopic fingerprint is recorded in the morphine (biological product), which contributes 17 of the 21 carbon atoms and all of the nitrogen atoms in heroin. The carbon isotope ratios of acetic anhydride will vary with the source of carbon used to synthesize the molecule (e.g., fossil fuel, biogenic, and syngas sources). Table 15.2 shows the carbon isotope ratios of a number of different DEA exhibits acquired in one region of the world. Note the similarity in values among different exhibits. In this case, the stable isotope ratio information was useful in further characterizing the acetic anhydride and allowing investigators to link different exhibits to indicate a common source.

Ehleringer et al. [4] showed that the major growing regions of coca for cocaine in South America could be characterized by different combinations of carbon and nitrogen isotopes (Figure 15.4). In this case, these two isotopes alone explained more than 80% of the observed variation among samples originating from different growing regions. This information is useful in law

TABLE 15.2 $\delta^{13}C$ Values (‰) of Morphine Base Obtained Under Controlled Conditions from Illicit Production by Clandestine Chemists in Colombia [8]

Chemist	Morphine Base Starting Material
Colombian—1	–32.1
Colombian—2	–32.0
Colombian—3	–32.2
Colombian—4	–32.3
Colombian—5	–32.1
Colombian—6	–32.2
Colombian—7	–32.2
Colombian—8	–32.3
Colombian—9	–31.9
Colombian—10	–31.9
Colombian—11	–32.4
Colombian—12	–32.2
Average	–32.2

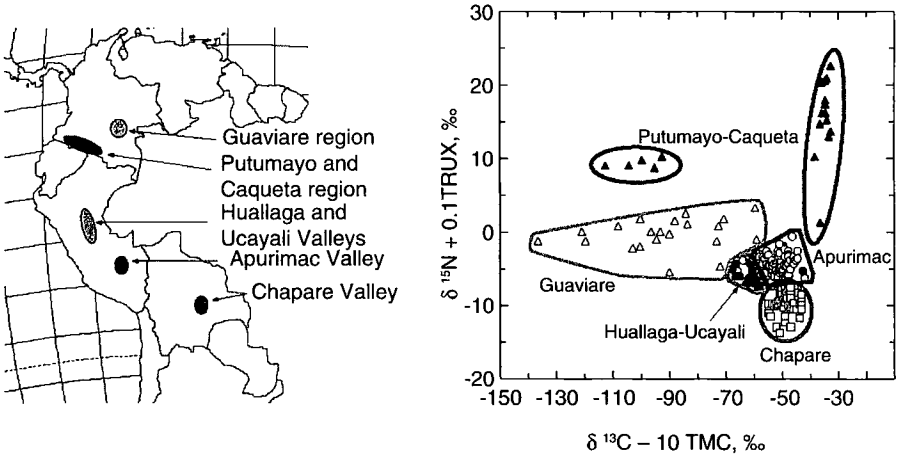

Figure 15.4 *Carbon and nitrogen stable isotope ratios allowed the correct identification of country of origin of 90% of 200 cocaine samples analyzed (from Bolivia, Colombia, or Peru). Including the abundance of minor alkaloid compounds allowed further separation of growing regions within countries and increased the accuracy of country identification to 96%. After [4]*

enforcement for strategic and intelligence purposes. Today stable isotope analyses are a key measurement in the U.S. DEA's Cocaine Signature Program, providing critical information about the origins of seized samples.

Recently, stable isotope ratio analyses were used to comment on the origins of heroin samples seized on the freighter *Pong Su*, a ship sailing under North Korean registry [8, 36]. Australian police seized 50 kg of heroin hydrochloride from the freighter and another 75 kg of heroin hydrochloride from the offload site on Australian soil. Authorities believed that it was "highly likely" that

North Korea was dealing in illegal drugs [37]. Stable isotope ratio analyses of the samples were conducted to examine their region of origin. Using authentic heroin exhibits, Ehleringer et al. [35] had earlier established that the four major growing regions for heroin (SE Asia, SW Asia, Mexico, and South America) could be distinguished on the basis of their carbon and nitrogen isotope ratios. Casale et al. [8] completed stable isotope ratio analyses of both the seized samples and deacetylated seized samples; they concluded that the seized samples were unlike any previously known heroin exhibits and that the samples were unlikely to have originated from any of the four major known growing regions.

Denton et al. [38] and Shibuya et al. [39] have shown that variations in the carbon and nitrogen isotope ratios have been useful in detecting regions of origin for marijuana seizures in Australia and Brazil, respectively. The observations by Shibuya et al. [39] were particularly useful as they provided police with information about the geographic origins of street sales in Sao Paulo, Brazil, allowing law enforcement to better focus their efforts and reconstruct trafficking routes.

Synthetic drugs such as ecstasy can also exhibit stable isotope ratio differences that are useful to law enforcement for determining relationships among batches of seized samples. Carter et al. [40] showed that, by plotting different combinations of the hydrogen, carbon, and nitrogen isotope ratios of seized ecstasy tablets, it was possible to identify clusters or groupings reflecting different production batches. This "isotopic fingerprint" allowed investigators to be able to link specific batches of the illicit drug with particular manufacturers. Palhol et al. [41] showed that is was possible to distinguish among and relate seizure samples of ecstasy originating from different geographic locations based on their nitrogen isotope ratio values. In both of these cases, the combinations of stable isotope ratios does not provide information about the origins of the seized samples, but instead allows the investigator to know how many different "cooks" or batches contributed to the samples that were seized.

Doping is a significant concern in both professional and amateur sports [42] and has resulted in the formation of organizations such as the World Anti-Doping Agency (WADA; www.wada-ama.org) with the intent to fight doping in athletics [43]. The ratio of testosterone to epitestosterone in urine can indicate synthetic steroid use and has been used to test for their use. However, because of the identification of naturally high T/E ratios in some individuals and the need to monitor athletes over time to confirm a return to a baseline condition, carbon isotope ratio analysis has been explored as an additional tool for detection [44]. Carbon isotope ratios of synthetic steroids provide a marker of steroid use because synthetic steroids are derived directly from plant sources and therefore tend to have lower $\delta^{13}C$ values [45, 46]. The use of synthetic steroids by an athlete would therefore be detected by lower $\delta^{13}C$ values in urinary steroids than those for one who has not. However, this value alone can be affected by diet and potentially other factors [47]. By comparing the $\delta^{13}C$ of endogenous steroids unaffected by synthetic inputs with those

potentially derived from synthetic sources in urine samples, it is possible to unequivocally identify exogenous steroids since, if no synthetic steroids were used, their $\delta^{13}C$ should not be substantially different [47, 48].

15.6.3 Sourcing of Humans, Animals, and Animal Products

The isotopic composition of an organism will reflect the results of its dietary inputs through food and drinking water [2, 49, 50]. Large differences in the carbon isotope ratios of C_3 plants ranging from –30‰ to –24‰ (e.g., wheat, barley, potatoes) and C_4 plants ranging from –14‰ to –10‰ (e.g., corn, millet, sorghum, sugarcane) result in contrasting dietary inputs [10]. Assimilated dietary source inputs get laid down in proteins, lipids, carbonates, and carbohydrates of the muscle, bones, teeth, and hair of organisms, providing a record of the diet of that organism. Hair provides a chronological recording of the diet of an organism, be it a traveling human [51] or a migrating animal such as an elephant [52]. Naturally occurring variations in both light (H, C, N, and S) and heavy (Pb, Sr) isotopes have been used to associate humans and animals with specific geographic regions of the world. Variations in C, N, and S isotopes largely reflect dietary factors [2, 52, 53], while H isotope variations have been related to geographic patterns of water isotopes across the landscape [2, 9, 54, 55]. Within the heavy isotopes, both Pb and Sr have been shown to provide provenance and source information because these isotopes are picked up from local sources via dietary inputs [56–58].

Geographic variations in the hydrogen and oxygen isotope ratios of water form a basis of a geographic signal recorded in the hydrogen and oxygen isotope ratios of organic matter in animals. Bowen and colleagues [9, 59, 60] have been able to extrapolate from the available location-specific data of stable isotopes in water to provide spatial maps of the predicted isotopic composition of water throughout the world. Analyses of the spatial distributions of hydrogen isotopes of waters across the North America and Europe continents reveal substantial variations in stable isotope ratios (Figure 15.5), making it possible to distinguish many geographical locations. There are not unique stable isotope ratio values for waters in a specific geographic location, but rather gradients or bands of different isotope ratio values allowing one to distinguish between locations if they were sufficiently far apart from each other.

Kreuzer-Martin et al. [61, 62] have applied this known variation in the isotope ratios of water to show that it is possible to source microbial spores, such as the anthrax spores mailed in letters in late 2001 (http://en.wikipedia.org/wiki/2001_anthrax_attacks). The hydrogen and oxygen isotope ratios of spore cell wall materials record the isotopic composition of the water in which these microbes were cultured, providing a geolocation piece of information. This source-location information is preserved as long as the original spores remain intact. In *Bacillus* spores, approximately 74% of the oxygen atoms in a spore were derived from its source water (Figure 15.6). The utility of the stable isotope approach here is to allow investigators to both eliminate

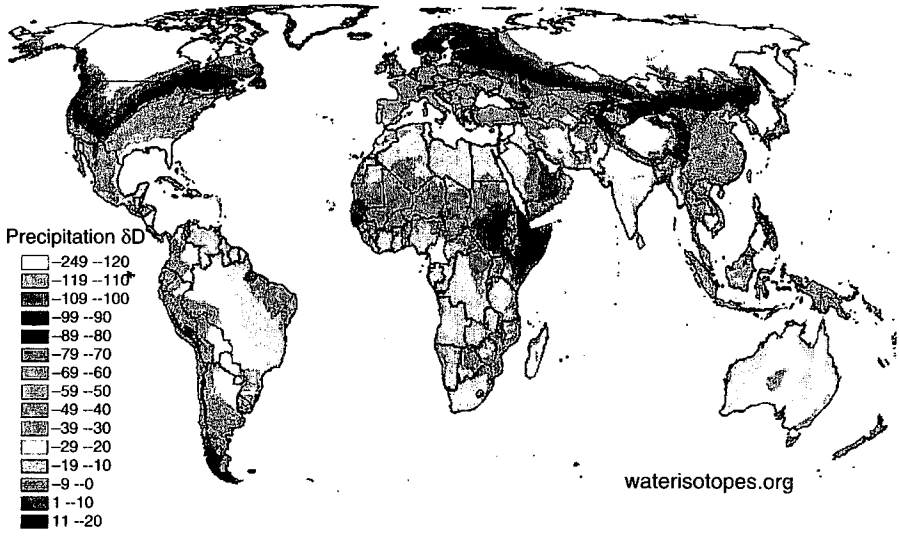

Figure 15.5 *(See color insert.) Predicted long-term annual average precipitation hydrogen isotope ratios for the land surface. This continuous layer is produced with a combination of empirical relationships between measured precipitation δ^2H and latitude and elevation, and a geostatistical smoothing algorithm for variation not explained by that relationship. Measured precipitation values are those maintained in the International Atomic Energy Agency (IAEA) water isotope database. Methods and grids are available at http://waterisotopes.org.*

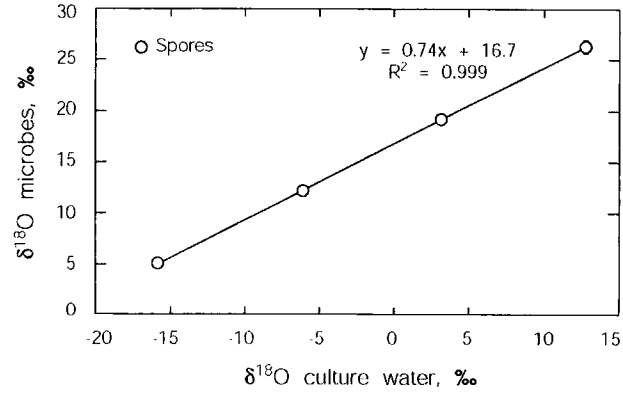

Figure 15.6 *The oxygen isotope ratios of Bacillus spores are tightly coupled to the oxygen isotope ratios of the water in which they are cultured. As can be seen in the fitted regression equation, approximately 74% of the oxygen in these bacterial spores was derived from the culture water. This oxygen isotope ratio signal allows clear matching between spores and their origins. After [61]*

possible sources by showing that stable isotope values are not consistent with a region and to further guide investigations by indicating that stable isotope ratio values are consistent with a different region. Similarly, the carbon and nitrogen isotope ratios of the spore provide information about the nature of the nutrient media in which a microbe is grown [62–64].

15.6.4 Humans: Bones, Hair, and Teeth

Trapped within the enamel of teeth are a number of trace components, including lead, strontium, and carbonates. Each of these components has isotope ratio variation resulting in four isotopes (C, O, Pb, Sr) that then provide geographic and dietary information to the forensic investigator. The carbon isotopes reveal dietary information from the time period when the tooth was originally formed (preadult), allowing one to gather general information about the role of C_3 versus C_4 plants in the diet [65, 66]. The oxygen in the carbonate reflects the oxygen of the water in blood, which is related to the geographical variations in drinking water [67]. Once deposited, the carbon and oxygen isotope ratios of teeth do not shift during a human's lifetime.

Carbon isotope and nitrogen isotope analyses of collagen in bone have been used to reconstruct dietary histories of both recent and prehistoric humans [68–71]. Collagen is a protein preserved in bones that reflects dietary inputs over a several year period, because of its slow turnover rate. Once preserved, collagen degrades very slowly over time, allowing for dietary reconstructions of both modern and ancient bone samples. In the anthropological arena, Ambrose and colleagues (as well as other investigators) have used carbon isotope analyses to reconstruct the importance of corn in ancestral Native American populations over the past millennium [72–74]. Richards et al. [75] recently examined dietary changes in Iron Age, Roman, and post-Roman bodies recovered from grave sites in Dorchester, England using stable isotope analyses.

The heavier element Sr shows geographic variation related to soil differences in different regions and countries [58]. Sr is taken up by plants and makes its way into the food chain, becoming incorporated into humans as part of our diet. The Sr signal then provides a tool for reconstructing the geographic origins or movements of individuals across terrains. Evans et al. [76] used this information to trace origins of individuals. Price et al. [77] found that Sr in both teeth and bones could be used to distinguish migrants versus locals in a population. Gulson et al. [57] found that the lead isotope composition of human teeth from individuals living in eastern and southern Europe was significantly different from those individuals raised in Australia. Moreover, they were able to distinguish individuals raised in different portions of the Middle East, eastern Europe, and western Europe based on the lead isotope ratios of an individual's teeth. Pye and Croft [78] review the utility of

additional geological heavy isotope signals in a number of different forensic investigations.

The stable isotope ratios of human hair provide a recorder of human diet. Keratin is the dominant protein in hair and records information about both the carbohydrate and protein sources consumed by individuals. Perhaps most well documented are the changes in the carbon and nitrogen isotope ratios of hair related to geographically distinct dietary preferences [79]. Marine versus terrestrial diets are apparent [80] as are C_3-dominated versus C_4-dominated diets. European hair is distinct from U.S. hair, as is Chilean, Canadian, and German hair [81, 82].

Human hair and fingernails record dietary and water source information, and in so doing provide geographic information for forensic studies. Several recent studies have suggested that isotope ratio differences in human hair can be used to distinguish individuals of different geographic origin [83–85]. A recent study of public interest involving stable isotopes was the case of the Ice Man discovered on the border between Austria and Italy [86]. Fingernails are composed of keratin, the same protein found in hair. Recently, Nardoto et al. [79] have shown that citizens from Europe, the United States, and Brazil can be distinguished based on the isotope ratios within their fingernails. Although there is a tendency among modern human societies for a global supermarket that would homogenize isotopes in fingernails, the diets in these countries are sufficiently distinct so as to create differences in the isotope ratios of human finger~~prints~~nails.

15.6.5 Stable Isotope Abundances of Manufactured Items

Manufactured or synthesized products of forensic interest can exhibit significant variation in their isotope ratios because of two manufacturing factors: differences in the substrates used to synthesize the product and differences in the manufacturing process. These materials span a broad array of items, including security papers, counterfeit currencies, plastic tapes, packaging materials, explosives, clothing, and synthetic drugs.

Consider the example of explosive compounds. PETN, RDX, TNT, and HMX are among the most common high-energy military explosives associated with terrorist events. On the other hand, ammonium nitrate, fuel oil, and pyrotechnic materials are often more common explosive materials used at the local levels. Both of these classes of explosive materials have been considered in forensic investigations in both the United States and the European Union in terms of all elements measured with an IRMS. Of significance is that explosive materials should be investigated through analysis of individual explosive compounds and not as analysis of the bulk explosive mixture (e.g., separate explosive compound binders, fillers, etc.). The left plate of Figure 15.7 shows two examples of common military explosives developed and manufactured in different countries, consisting of either PETN and RDX or TNT and RTX; the names of the manufacturers are eliminated to maintain anonymity. In both

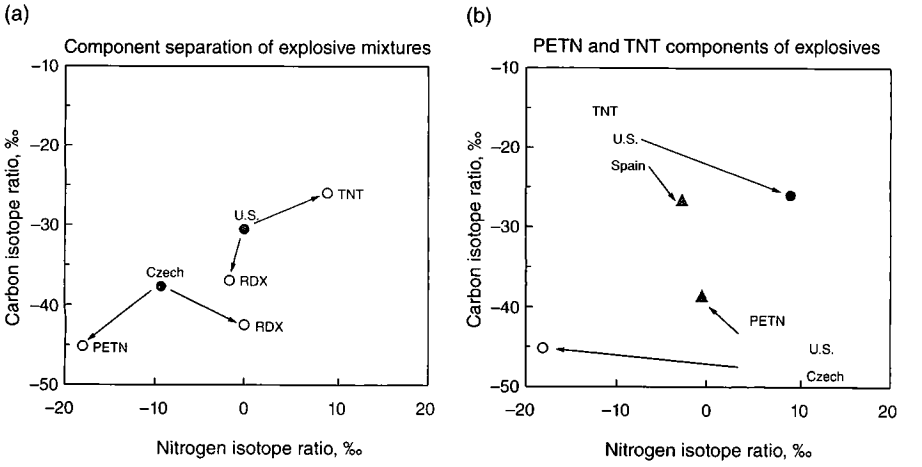

Figure 15.7 *Explosives have distinct carbon and nitrogen isotopic signatures, depending on the manufacturing process and materials used. This can be seen in the left panel showing Czech and U.S. explosives clearly separated by their $\delta^{13}C$ and $\delta^{15}N$ values. Separating these explosives into their component parts and analyzing the component isotope ratios yields even greater distinction based on differences in these component isotope ratios.*

cases, the explosive mixture is composed of two explosive compounds with very different carbon–nitrogen isotope ratio combinations. When considered as individual compounds, the isotope ratio combinations comprise a "fingerprint" that characterizes the explosive material, allowing that explosive to be distinguished from other mixtures of the same chemical composition. Applications include comparisons of seized materials and efforts to link explosive materials through a series of connected sources. This difference in isotope ratio "fingerprints" is made even more evident when investigators look at the carbon–nitrogen isotope ratio combinations of pure compounds originating from different factories (here noted as different countries in Figure 15.7). PETN manufactured in the United States or Czech Republic are isotopically identifiable, whereas using traditional GC and LC preparation techniques the explosives would not be distinguishable from each other. The same analytical approach applies to distinguishing TNT and RDX originating from different factories (expressed as countries in Figure 15.7). This new analytical approach opens new opportunities in forensic science that allow investigators to distinguish among compounds that might otherwise appear as identical using traditional analytic methods.

Consider next the possibilities associated with multiple origins of or the counterfeiting of commercial products. Pharmaceutical drugs, security paper, perfumes, and other profitable items fall into this category. This situation is analogous to adulteration of biological products discussed in an earlier section.

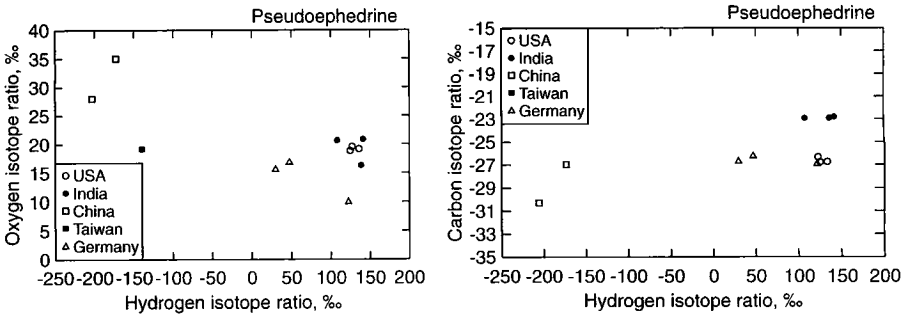

Figure 15.8 *$\delta^{13}C$, $\delta^{2}H$, and $\delta^{18}O$ values of pseudoephedrines of different geographic origins. Samples provided courtesy of Mexican Customs and reported by Lott et al. [7].*

In this case, the forensic investigation may require an understanding of the origins of a particular material, such as with explosives as just discussed. Here stable isotopes might provide sufficient insights that allow an investigator to trace the manufacturing origins of the material at hand. Figure 15.8 shows the isotope ratio combinations for pseudoephedrines that might be imported without duties into Mexico from the United States as part of NAFTA [7]. The importing of such materials that did not originate from the United States could be detected through isotope ratio analysis. Figure 15.8 shows the carbon, hydrogen, and oxygen isotope ratios of materials manufactured in different countries using different synthetic processes. Note that the geographic origins of the materials can be detected in this case on the basis of certain isotope ratio combinations. Although the pseudoephedrines of different origins do cluster on some axes, they are separated and identifiable using other axes. In this case, stable isotope analyses can be used in screening to distinguish legitimate from illegitimate samples; or possibly to track down the origins of counterfeited sample materials.

There is no exhaustive list yet of the classes of studies for which isotope ratio analyses will or will not be useful for forensic investigations. However, it is clear that in some cases, direct sample comparisons will be used to determine whether or not two samples have a common origin (i.e., possibly coming from the same production batch). In most cases, law enforcement interests will need to develop databases of observations from different sources and repeated sampling over time in order to determine the general usefulness of stable isotopes in forensic studies.

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