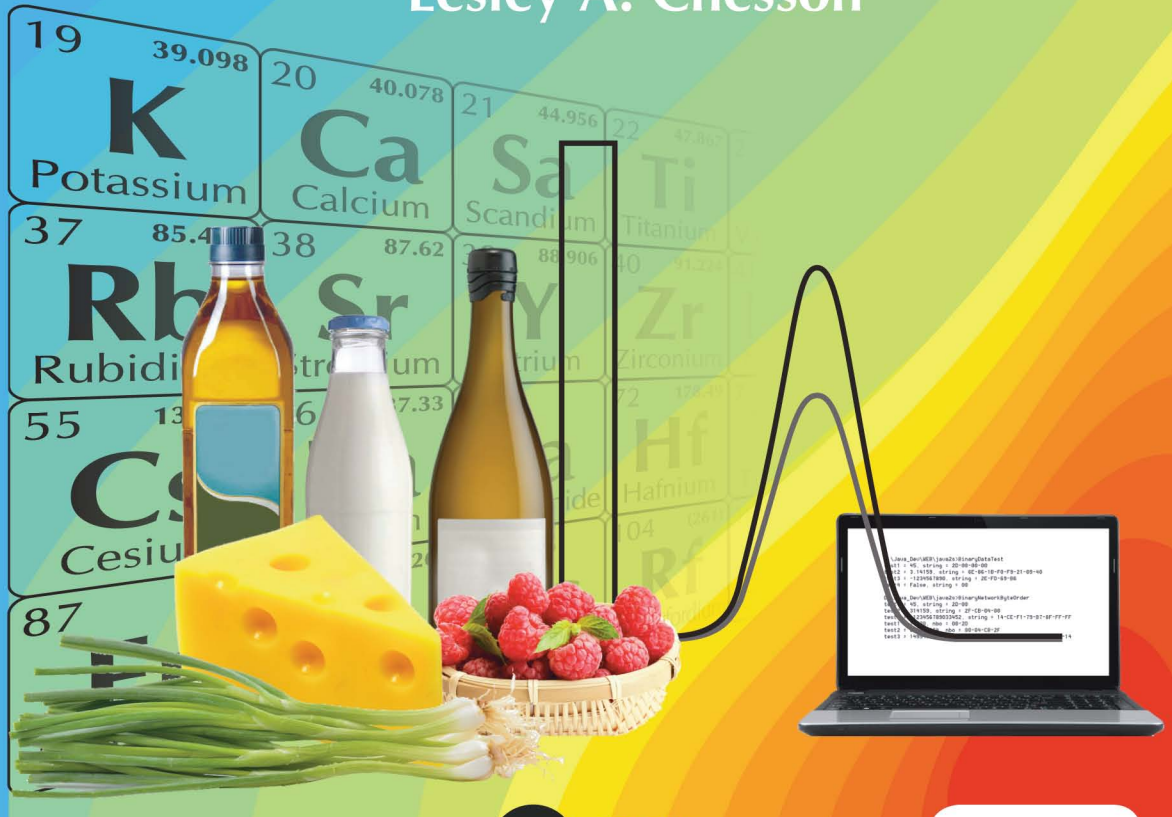
Food Forensics

Stable Isotopes as a Guide to Authenticity and Origin

Editors
James F. Carter
Lesley A. Chesson



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Foreword

The horse meat scandal of 2013 refocused global attention on food authenticity and origin and the harm that food fraud inflicts. Consumers are duped into spending money that some can ill afford, trust is lost and business reputations are damaged. Food safety is also compromised, for example the deaths that have arisen from counterfeit alcohol products. The subsequent Review by Professor Elliott*, in which I was privileged to play a part, described how 'cutting corners' elides through food fraud into '*food crime*', an organised activity by groups which knowingly set out to deceive, and/or injure, those purchasing food. The UK Government accepted the Elliott recommendations, including setting up National Food Crime Units within the Food Standards Agency and Food Standards Scotland. These Units' 2016 baseline threat assessment was reassuring in that it found that organized crime groups have not made substantial in-roads into UK food and drink in the way they have in other countries. However, more worryingly, a small number of UK food businesses are believed to have links to organized crime.

Within the systems approach based on eight pillars of food integrity advocated by the Elliott Review laboratory services is a key component. Stable isotope analysis was one of the techniques Professor Elliott and I considered. We were anxious that the promise of this demanding technique, reaching maturity in its mainstream criminal forensic applications, should not be compromised by lack of forensic probity in its application to food.

Hence ***Food forensics: stable isotopes as a guide to authenticity and origin*** edited by Jim Carter and Lesley Chesson is timely and valuable. This volume, the first to focus exclusively on food authentication and geo-location by stable isotope analysis and allied techniques, provides a sound basis to their scientific practice with forensic rigor. The international contributors are all acknowledged experts in their fields.

The topics covered appropriately commence with an introduction to stable isotopes, reference materials, traceability, scientific good practice and reporting. Since they are essential to the proper forensic application of any technique it is pleasing to see chapters on sampling and sample preparation of foodstuffs and other forensic exhibits as well as quality

assurance and control of data, data analysis and interpretation. The latter, drawing on international criminal forensic standards includes common statistical approaches for exploring food isotopic data alongside examples of investigating food authenticity and origin.

Well-established theories to explain variations in the natural abundances of hydrogen, carbon, and oxygen isotopes in plants are dealt with in a dedicated chapter providing the food scientist with sufficient information to predict variations in the isotopes of these elements in plant-based foods. Interpretations of variations in nitrogen, sulfur, and strontium isotope ratios of plant and plant-based foods are also discussed. There are separate chapters on flesh foods, fruits and vegetables, alcoholic beverages, dairy products, and edible oils. The chapter on food webs, the detailed ecological history of an organism and its relationship with other organisms on different time scales, shows how stable isotope analysis answers questions such as where was the animal living and what was it eating when its tissue was formed? While some techniques may be challenged in distinguishing conventional food from food described as 'organic' emerging compound-specific isotope techniques, in combination with other multi-marker strategies and multivariate statistical tests, are more promising, as described in a chapter dedicated to organic food. A final chapter deals with miscellaneous topics such as bottled water, carbonated soft drinks, caffeine, vanilla/vanillin, essential oils, sweeteners, honey, maple syrup and other food products as well as isotope effects during food preparation.

The passion of the editors and authors, who form a distinguished scientific community, contributes to the breadth of coverage of this book, the depth of each chapter, and its readability. Thus the book will serve as an informative reference text for new users as well as a reminder of best practice for seasoned professionals. I congratulate the editors and contributors for successfully compiling this excellent volume that describes the state of the art of a valuable scientific asset for the detection and prevention of food fraud and food crime.

Dr. Michael Walker MChemA, FIFST, FRSC

Former Public Analyst, now Referee Analyst in the Laboratory of the Government Chemist, UK and Subject Matter Expert to the Elliott Review.

* Elliot C. 2014. Elliot review into the integrity and assurance of food supply networks—Final report—A national food crime prevention framework. Edited by Food and Rural Affairs Department for Environment and Food Standards Agency: HM Government.

Michael Walker

Preface

Food forensics is a science that seeks to determine the authenticity and origins of (typically premium) foodstuffs using techniques more commonly applied in high profile criminal forensics. The science of food forensics can be traced back many centuries and has always used cutting-edge technology to try and remain "one step ahead" of the knowledgeable fraudster. Because sophisticated fraudsters can often, convincingly mimic the physical and chemical compositions of targeted foods, a current tool of choice in food forensics is stable isotope analysis.

The invisible "signature" or "fingerprint" inherent in the stable isotope ratios of bio-elements (hydrogen, carbon, nitrogen, oxygen, and sulfur) is often combined with elemental concentration data and the isotope ratios of trace radiogenic elements, such as strontium and lead. The complexity of this isotopic "signature" is impossible to detect without access to highly sophisticated analytical instrumentation and is extremely difficult to counterfeit without a detailed knowledge of numerous physical, chemical, and biological processes that can affect isotope abundances in foodstuffs. Food forensics is a science that draws on many, diverse disciplines, including plant and animal biology, hydrology, analytical chemistry, and statistics.

An understanding of plant and animal nutrition and metabolism is important to understanding and interpreting the ways in which stable isotopes are assimilated by plants and the animals consuming these plants. Through this understanding it is possible to interpret the isotopic "signature" and to infer the cultivation practices and origins of plants and the diets and origins of animals. This volume presents current, widely-accepted theories on the processes that impact food isotopic "signatures" and the interpretations of food source an investigator may be able to draw based on those theories.

A cornerstone of forensic science is that both raw analytical data, and any interpretation based on these data, will withstand scrutiny in a court of law. An understanding of analytical chemistry is also essential to generate "forensic" data, which must include an understanding of the need for traceability, quality control, and quality assurance. The analysis of large, complex datasets requires sophisticated statistical tools and when

interpretations based on such data are to be presented to a court of law, it is essential that statistical treatments are both appropriate and valid. It is often desirable that large, complex datasets can be presented in a simple graphical form that can be presented to a layperson serving on a jury. As described throughout this volume, stable isotope analysis, along with allied techniques, supplies quantitative data that is well-suited for forensic interpretation, in both investigative and court settings.

This book draws together many diverse branches of science, and many internationally renowned scientists, to present *food forensics*: stable isotope analysis (and allied techniques) as a guide to the origin and authenticity of the most common (and not-so-common) foodstuffs.

James F. Carter
Lesley A. Chesson

Acknowledgements

On reflection, I spent too long writing the introduction to this volume—thinking it would be the chapter that most people would read, but writing this acknowledgement proved more difficult, principally because it marks the end of a long but ultimately very rewarding process. I am now faced with the questions of who to acknowledge, for what, and in what order.

There is no question that the first person I must thank is my beloved wife, Gabrielle, for her endless patience and encouragement (especially through some recent bad times) and for allowing me to spend most evenings of 2016 writing and editing this volume. I must also thank Caroline Vouvoulis for being the most wonderful step-daughter and for all the trust she puts in me.

Next (and again without question) I must thank Lesley for the boundless energy she expended on this project. Clearly this project sparked our unlikely mutual passions for food chemistry and stable isotopes and what began as a vague idea of "putting a book together with some friends" turned into something real. I must also thank Lesley for teaching me American English (of which "liters" was the most painful lesson) and for our regular exchanges of emails that kept me sane although some might argue otherwise.

I am, of course, indebted to all the contributors to this volume and I am honestly surprised that they responded to my initial e-mails, let alone spent time and effort to share their knowledge. Writing/editing this volume was a wonderful interaction between the various authors—everyone worked when their input was needed, everyone respected other's input, and no-one pushed themself to the front. Thanks to all of you.

I would very much like to acknowledge the UK Association of Public Analysts (APA) for the great insight I gained from attending their training sessions and summer schools. I don't think this volume would exist without the inspiration and knowledge I took away from those courses. It is wholly appropriate that the preface to this volume is written by one of the mentors for the APA.

Appropriately as a footnote, I would like to thank the Information Research Services (Library) at Queensland Health Forensic and Scientific Services for helping me cite some of the more unusual works on food authentication.

Dr. Jim Carter
(December 2016)

First, I need to extend a huge "thank you" to Jim Carter for the invitation to join him on this editing adventure. Having never edited a volume before, I was very glad to muddle through the process with Jim, whose logical and pragmatic approach and dry sense of humor about the missteps and setbacks we encountered helped the project go more smoothly than I could have ever imagined. I'd do it again, Jim… but perhaps not right away!

I was thrilled when I first learned of this volume, because it encompassed many of the things I'm passionate about—food and forensics and isotopes. Since finishing my master's degree in 2009, there had been relatively few opportunities for me to sit and think critically about these topics all at once. I enjoyed the chance to delve into the published literature and contribute to some chapters myself.

However, the truth is that this volume would simply not have been possible without the other chapter contributors. Several I knew personally and had worked with in the past; others I knew by professional reputation only. I feel privileged to have had the opportunity to work with them all. You would be hard-pressed to find a more experienced and better-qualified group of professionals to discuss the applications of stable isotope analysis to food forensics. It was an absolute honor to have them contribute works to this volume.

I am extremely appreciative of IsoForensics, Inc. and my colleagues/friends at the company. Thank you for your patience, support, and listening ears during the publication process. It meant a lot to me.

On the topic of "listening ears," I am profoundly grateful to my husband (Mike) and my parents (Boon and Mary) for their unflagging encouragement and enthusiasm—not just in the 3+ years it took to publish this volume, but for all the phases of my professional and personal life. Thanks for being there, always.

Finally, I want to thank the readers—I hope you will find this volume as interesting and valuable as I do. I look forward to hearing your thoughts. Thanks in advance for your positive response and feedback.

Lesley A. Chesson
IsoForensics, Inc.
(November 2016)

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CHAPTER 1

Isotope Ratio Measurements for Food Forensics

James F. Carter

1.1 Introduction

1.1.1 A bit of introduction...

> "To such perfection of ingenuity has the system of counterfeiting and adulterating various commodities of life arrived in this country, that spurious articles are every where to be found in the market, made up so skilfully, as to elude the discrimination of the most experienced judges."
>
> *Frederick Accum*

Although the words above were written in 1822, in the first edition of Accum's *Treatise on Adulterations of Food and Culinary Poisons* (Accum 1820), they provide a fitting opening to a modern text on food fraud. Indeed, even two centuries ago Accum went on to record "*... it is evident that this practice has been carried on for a long time*". Today, bogus goods are purchased (both willingly and unknowingly) on a daily basis, ranging from fripperies such as designer jeans and DVDs to items that may, very likely, make a difference between life or death—pharmaceuticals, aircraft components, etc. Although the true extent of this market can never be known, various sources across the Internet estimate that somewhere in the region of 10% of all traded goods are dishonestly presented for sale in some way (UK Government Scientific Advisor 2015).

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What Accum described as *nefarious practice* can be summarized as:

> *Counterfeiting*—the practice of manufacturing goods, typically of inferior quality, and selling them as a premium product without authorization.
> *Substitution*—the practice of presenting an inferior product as a premium product.
> *Adulteration*—the practice of diluting a premium product with an inferior product or with substances intended to mimic the product.

These terms are frequently used inter-changeably and may well be used as such in the following chapters. Often viewed as simply supplying a harmless "black" or "grey" market in which the none-too-fussy shopper can obtain seemingly "luxury goods" at "knock-down prices," the sale and purchase of counterfeit, substitute, or adulterated goods, especially foodstuffs, compromise both manufacturers and consumers through poor brand experience, loss of brand reputation, financial loss, and danger to health. The criminal nature of such practices gives rise to legislation intended for the general protection for purchasers of food:

> *If a person sells to the purchaser's prejudice any food which is not (a) of the nature, or (b) of the substance, or (c) of the quality, of the food demanded by the purchaser, he is guilty of an offence.*

The text above forms part of the UK Food Act 1984 but almost all countries of the world have similar legislation. In most cases legislation distinguishes between offences involving deception and those deemed to be more serious, such as the intentional or neglectful preparation and/or sale of injurious foods.

The manufacturers and retailers of many products, not just foodstuffs, now adopt a range of easily recognized devices to deter both potential counterfeiters and persons intent on deliberate and harmful contamination:

> *Sophisticated products*—products which are themselves difficult to copy.
> *Sophisticated packaging*—packaging with fancy designs incorporating devices such as holographic images and metallic strips.
> *Tamper evident packaging*—packaging which cannot be opened without leaving some obvious record.

Although such devices are primarily intended to reassure customers, the use of blatant product sophistication and garish designs can also serve to advertise a product—a practice far more common in designer goods such as sports shoes (trainers or sneakers) than foodstuffs. Like any new technology, anti-counterfeiting measures will be expensive to implement but difficult to copy. Unfortunately, as technology becomes readily available, the price inevitably falls and counterfeiters are able to adopt the same techniques. In

an effort to stay at least one step ahead of ever-sophisticated counterfeiters, food scientists are looking to intrinsic and difficult-to-copy properties of foods for authentication.

Many global organisations, national governments, and manufacturing associations have defined the physical and chemical properties of foodstuffs, by means of legislation, standards, and codes-of-practice. Although the primary function of these regulations is to ensure that food is safe and wholesome through defined composition, they can also provide a means to guarantee the authenticity of the product including the Country of Origin (CoO). Two properties of special interest for foodstuffs are the stable isotopic and elemental compositions of natural and manufactured products, which are controlled by a large number of parameters:

> *geography and climate*—will affect rainfall and temperature and hence the localized hydrogen and oxygen isotopic composition of water,
> *underlying geology* and *agricultural practices*—will affect nutrient availability from soil (nitrogen, micro- and macro-elements) to both plants and animals, and
> *manufacturing processes*—may introduce or remove components of a food and/or change the physical or chemical nature of some or all of these components.

The combined effects of these, and many other possible processes, gives rise to a *signature*, *fingerprint*, *footprint* or *profile* for a food product, which can be highly characteristic and which has been compared to the specificity of DNA analysis (Meier-Augenstein 2006). Stable isotope analysis, often combined with trace elemental profiling and chemical composition measurements, is at the forefront of *food forensics*, a discipline that seeks to use modern analytical techniques and sophisticated statistical tools to establish the authenticity and origin of foods.

1.1.2 A bit of history...

In 1913, Frederick Soddy, working at the University of Glasgow, discovered that the radioactive decay of elements such as thorium and uranium produced substances that exhibited chemical properties identical to those of known elements, such as lead. To account for these findings Soddy used the word *isotope* (meaning "same place") to explain what appeared to be different chemical elements occupying the *same place* in the periodic table. The origin of the word *isotope* is often attributed to a family friend of Soddy, Margaret Todd.

During the same rich period of experimental physics, both J. J. Thompson and Francis Aston, working at the Cavendish laboratories in Cambridge, observed the existence of naturally occurring isotopes of neon, using instruments which were soon to be known as *mass spectrometers*.

The combined importance of isotopes and mass spectrometers to modern science was acknowledged by Thompson's Nobel Prize for physics in 1906, Soddy's Prize for chemistry in 1921 and Aston's Prize for chemistry in 1922.

1.1.3 A bit of theory...

Atomic nuclei (*nuclides*) are characterized by:

> *atomic number Z*—the number of positively charged protons and,
> *atomic mass number A*—the total number of nucleons (protons + neutrons).

The information needed to describe an isotope is easily (and universally) summarized by the combination of the chemical symbol (equivalent to the atomic number) and the atomic mass number in the form; ^{11}B, ^{12}C, ^{20}Ne, ^{56}Fe, ^{208}Pb, etc. Every known element exists as a number of isotopes, both naturally occurring and man-made atoms of the same element with varying numbers of neutrons such as ^{10}B, ^{13}C, ^{22}Ne, ^{54}Fe, ^{206}Pb, etc. Importantly, changes in the nucleus have a subtle effect on the chemistry of the orbiting electrons, which, in part, explains why analysis of isotopic composition can be a powerful tool to study the origin and fate of many materials—sometimes termed *isotope chemistry*.

Isotopes are classed as either *stable isotopes* or *radio-nuclides* (note that a greater or lesser number of neutrons does not necessarily make a nucleus more or less stable). The distinction is that stable isotopes endure over geological time scales, whereas radio-nuclides have characteristic half-lives ($t_{½}$) and spontaneously transform into stable isotopes through the emission of radioactive particles. The stable products of radioactive decay, such as ^{87}Sr, ^{206}Pb, and ^{208}Pb, are often described as *radiogenic* isotopes and were the original subject of Soddy's experiments.

To demonstrate some of these ideas, consider carbon, which exists as 15 known isotopic forms but predominantly as two stable isotopes: ^{12}C and ^{13}C, with relative abundances of approximately 98.9 and 1.1%. The ratio of these isotopes has not changed since the Earth was formed, although localized variations occur as a result of numerous physical, chemical, and biological processes. To record a stable isotopic *fingerprint* simply requires a very precise measurement of the $^{13}C/^{12}C$ ratio of a sample. In contrast, the next most abundant form of carbon, ^{14}C, is continuously formed in the Earth's upper atmosphere in very small amounts—$^{14}C/(^{12}C + ^{13}C)$ approximately 10^{-12}. All three forms of carbon are assimilated into plants, animals, and minerals to become part of the carbon *isotopic signature*. The ratio of stable isotopes $^{13}C/^{12}C$ is largely controlled by plant metabolism and/or animal diet and is frequently used to interpret these conditions. The radioactive form of carbon decays with a predictable

half-life ($t_{½}$ = 5,730 yrs) and the residual ^{14}C activity provides a useful means by which to estimate the age of materials over an approximate 1,000 to 10,000 years (from present).

As an aside: the 1950s and 1960s witnessed a significant increase in the concentration of atmospheric ^{14}C as a result of nuclear weapons testing—the *bomb-pulse.* The residual activity from these tests can be used to date biological materials post the mid-1950s with a reported ± 2-year resolution. Both conventional *radiocarbon* and *bomb-pulse* carbon dating have found forensic applications (Tuniz et al. 2004); for example, *bomb-pulse* carbon dating (Asenstorfer et al. 2011) and ^{137}Cs decay (Hubert et al. 2009) have been applied to authenticate vintage wines.

1.2 Reporting and reference materials

1.2.1 Reporting isotopic composition

Isotopic compositions can be expressed as either absolute or relative measurements, appropriate to particular applications. The following text strives to conform to the recommendations of the Commission on Isotopic Abundances and Atomic Weights (CIAAW) of the International Union of Pure and Applied Chemistry (IUPAC). For a comprehensive explanation of this sometimes bewildering nomenclature the reader is referred to Ty Coplen's comprehensive text on the subject (Coplen 2011).

When isotopically labelled materials are used, for example as tracers in metabolic studies, absolute values are determined; the *isotopic* or *radiochemical purity* is the fraction of the label present in a specified chemical form, the position within a molecule, or even the chiral form of the compound. *Radionuclidic purity* is the fraction of the total radioactivity present as a specified radionuclide and conveys nothing about the chemical form. These macroscopic measurements are expressed as the *atom fraction* (x), the amount (n) of a specified isotope divided by the total amount of atoms of the element within the sample. For an element with two major isotopes, such as carbon, the atom fraction in a sample is expressed by Equation 1.1.

$$x(^{13}\mathrm{C}) = \left(\frac{n(^{13}\mathrm{C})}{n(^{12}C) + n(^{13}\mathrm{C})}\right) \qquad \text{Equation 1.1}$$

Atom fraction is a dimensionless quantity but mmol/mol, µmol/mol and similar units are acceptable to most peer reviewed journals. Use of the term *atom percent* (atom%) is no longer recommended.

The small variations in the natural abundances of stable isotopes of the bio-elements (H, C, N, O, and S), which are of interest in food forensics, are almost universally expressed as relative measurements. The important reason for this is that the precision with which differences can be measured will always better that the accuracy with which absolute values can be

measured (Hayes 2001). The *isotope ratio* (R) of a sample is the ratio of the number of atoms of one (heavier) isotope to the number of atoms of another (lighter) isotope of the same chemical element, Equation 1.2:

$$R(^{13}\mathrm{C}/^{12}\mathrm{C}) = \frac{N^{13}\mathrm{C}}{N^{12}\mathrm{C}} \qquad \text{Equation 1.2}$$

In Equations 1.1 and 1.2, the symbols n and N are used respectively to denote the amount of a given isotope on macroscopic and microscopic scales.

The widely accepted form of reporting isotopic composition (shown in Equation 1.3) is known as *delta-notation* and dates from 1948 when the journal *Science* reported a lecture by Harold Urey in which he expressed the oxygen isotopic composition of various marine carbonates relative to the shell of a sea snail (Urey 1948). Relative isotope-ratio differences, expressed as *delta*-values, δ-values or simply δ can be the difference between any two substances, but are almost universally reported relative to an international reference material (RM). For example, the δ-value for the carbon isotopic composition of a sample (*samp*) is defined by:

$$\delta^{13}\mathrm{C}_{RM} = \left(\frac{R(^{13}C/^{12}C)_{samp} - R(^{13}C/^{12}C)_{RM}}{R(^{13}C/^{12}C)_{RM}} \right) \qquad \text{Equation 1.3}$$

The first published form of Equation 1.3 appeared discretely in a 1950 paper, which focused on electronic circuits for a new design of stable isotope ratio mass spectrometer (McKinney et al. 1950). The variations in isotopic abundances for most commonly studied chemical elements are typically small, and values are reported in part per hundred (% or percent), part per thousand (‰ or per mil) or part per million (ppm or permeg). It is no longer considered appropriate to include these fractions in Equation 1.3. Because of Urey's role in the genesis of δ-notation it has been proposed that the SI units of relative isotopic ratio be named the *Urey* (Brand and Coplen 2012), with the more common per mil replaced by milli-Urey (mUr).

In food forensics, and many other fields, it is often important to study the differences between the isotopic compositions of the various components of a system, for example between the carbon isotopic composition of a plant and the soil in which it grows. This can be conveniently expressed in Δ-notation. For the example above;

$$\Delta^{13}\mathrm{C}_{\mathrm{plant/soil}} = \delta^{13}\mathrm{C}_{\mathrm{plant}} - \delta^{13}\mathrm{C}_{\mathrm{soil}} \qquad \text{Equation 1.4}$$

Note that the delta symbol, both upper and lower case, is italicized. Also, since δ-values are difference measurements, authors are encouraged to prefix values greater than zero with the + symbol.

1.2.2 Traceability

The *take home message* from the previous section is that stable isotope abundance measurements are reported relative to reference materials (RMs) and for that reason the correct selection and use of RMs is the cornerstone of measurements that are both repeatable (within a laboratory) and reproducible (between laboratories).

All measurements reported in SI units should be traceable to a unique reference, which is accepted throughout the world. Most SI unit are now based on quantum phenomenon, although some are still based on artefacts such as the International Prototype of the Kilogram (IPK), a platinum/iridium cylinder, which defines at present the SI unit of mass. In the case of stable isotope abundance measurements, the *primary reference materials* fall very much under the heading of *artefact*. These materials define the zero point on the respective δ-scale—mostly!

The concept of traceability depends on a chain of measurements linked back to the appropriate international primary RM (artefact or quantum phenomenon) through a series of calibrations—i.e., comparisons between two materials in the chain. Provided the uncertainties of the comparisons are known, results obtained through calibration against one of these RMs will itself be traceable to the agreed reference. Table 1.1 lists the internationally agreed reporting scales and primary RMs for the five major elements in the biosphere. The RMs used for stable isotope ratio measurements can be confusing and the materials shown in Table 1.1 deserve some explanation.

Table 1.1 The primary reference materials for reporting isotope ratio measurements.

Element	Ratio	Reporting scale	Artefact	Current RM
Hydrogen	$^{2}H/^{1}H$	Vienna Standard Mean Ocean Water (VSMOW)	SMOW/ VSMOW water	VSMOW2 water, SLAP2 water
Carbon	$^{13}C/^{12}C$	Vienna PeeDee Belemnite (VPDB)	PDB PeeDee Belemnite	NBS-19 calcium carbonate, LSVEC lithium carbonate
Nitrogen	$^{15}N/^{14}N$	Atmospheric nitrogen (N_{2AIR})	Air	Air
Oxygen	$^{18}O/^{16}O$	Vienna Standard Mean Ocean Water (VSMOW)	SMOW/ VSMOW water	VSMOW2 water, SLAP2 water
Sulfur	$^{34}S/^{32}S$	Vienna Canyon Diablo Troilite (VCDT)	CDT Canyon Diablo Troilite	IAEA-S-1 silver sulfide

1.2.3 VSMOW

VSMOW was a physical sample of water prepared by the International Atomic Energy Agency (IAEA), which defined the zero point of the δ-scales for both hydrogen and oxygen measurements with no associated

uncertainty. VSMOW replaced an earlier RM, SMOW, which was both a physical sample of water and a defined isotopic composition. The prefix "V" was added to avoid further confusion. The original supply of VSMOW has now been exhausted and since 2009 has been replaced by VSMOW2, also with a value of 0.0‰. Because VSMOW2 has been calibrated against the original VSMOW, there is uncertainty associated with the composition, albeit very small (δ^2H ± 0.3‰ / δ^{18}O ± 0.02‰) and this uncertainty is likely to become smaller with subsequent use. The hydrogen and oxygen δ-scales have been further anchored with a second sample of water, Standard Light Antarctic Precipitation (SLAP2), such that $\delta^2H_{VSMOW\text{-}SLAP}$ of SLAP2 = –427.5‰ and $\delta^{18}O_{VSMOW\text{-}SLAP}$ of SLAP2 = –55.50‰, with the uncertainties given above.

1.2.4 VPDB

Like VSMOW, VPDB began as a physical material (PDB), a sample of calcium carbonate (limestone) derived from a belemnite fossil from the Pee Dee region of South Carolina, USA (hence PDB). This material was very quickly exhausted and was replaced by a defined value (Coplen 1994). The carbon isotopic composition of a material with δ^{13}C = 0‰ relative to VPDB is:

^{12}C = 0.988 944(28)

^{13}C = 0.011 056(28)

The values in brackets indicate the uncertainty in the final decimal places despite this being a defined value. Recognizing the limited practical value of this definition, the IAEA also defined the scale relative to another calcium carbonate material NBS-19 such that: $\delta^{13}C_{VPDB}$ of NBS-19 = +1.95‰ exactly.

1.2.5 N_{2AIR}

In many ways atmospheric nitrogen is an ideal RM for nitrogen isotopic composition as it is freely available and shows negligible global variation (Mariotti 1983; Mariotti 1984). The main disadvantage of atmospheric nitrogen as a RM is the difficulty in comparing a gaseous material with nitrogen evolved from solid samples. For convenience several solid reference materials (mostly ammonium and nitrate salts) have been prepared with δ-values ranging from approximately +180 to –30‰ *vs.* N_{2AIR}. Unfortunately, the assigned δ-values for all of these materials have relatively large uncertainties, typically ± 0.2‰.

1.2.6 VCDT

Canyon Diablo Troilite (CDT) was developed from meteorite fragments from the Barringer meteor crater (Canyon Diablo, Arizona, USA), which contained a number of minerals including troilite, a form of iron sulfide. For

any number of reasons choosing a Martian meteorite as the primary standard for terrestrial sulfur isotope abundance was not ideal but ultimately it was a lack of homogeneity (>0.4‰) that lead to the replacement of this material with a defined value (VCDT) by assigning IAEA-S-1 silver sulfide reference material $\delta^{34}S_{VCDT} = -0.3$‰ exactly (Krouse and Coplen 1997).

1.2.7 Practical reference materials

In practice a laboratory will rarely use primary RMs to perform routine stable isotope ratio measurements. The possible exceptions are hydrogen and oxygen stable isotope measurements of water samples for which VSMOW2 and SLAP2 are readily available and relatively inexpensive. Other primary material may be incompatible with the sample introduction system or simply unavailable. It is, however, vital that a laboratory obtains a range of primary and/or secondary RMs as the starting point for inter-laboratory comparability. Table 1.2 shows a typical hierarchy of RMs that should be available in a stable isotope laboratory.

Primary and secondary RMs will principally be used to calibrate a range of in-house RMs, which should be a close physical and chemical match for

Table 1.2 Typical hierarchy of reference material used for stable isotope ratio measurements.

RM Type	Description	Example	Comments	Uncertainty (1 standard deviation)
Primary	Physical materials which define the international δ-scales	NBS-19 IAEA-S-1	Limited supply—0.5 g available for purchase every 3 years; Impractical for day-to-day use	None
Secondary	Physical materials with internationally agreed δ-values and uncertainty	USGS-40 L-glutamic acid, IAEA-N-1 ammonium nitrate, IAEA-CH-7 polyethylene	Limited supply; Limited range of materials and δ-values	<0.05‰ $\delta^{13}C$ ~0.2‰ $\delta^{15}N$ ~ 2‰ $\delta^{2}H$
In-house	Materials calibrated against Primary and/or Secondary RMs		Used on a daily basis for QA and QC; Chemically and physically similar to typical laboratory samples	Ideally < 0.15‰ $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ < 2.0‰ $\delta^{2}H$
Working gas	Gas from high pressure cylinders		Calibrated daily against in-house RMs	

samples that are routinely analyzed at the facility. These in-house RMs will be used on a day-to-day basis for calibration and quality control (QC) and Quality Assurance (QA) (Carter and Fry 2013a). Individual samples are typically measured relative to laboratory working gases delivered from high pressure cylinders. The isotopic composition of these gases will change over time and must be calibrated against in-house RMs, ideally on a daily basis.

This chain of calibration will ensure that laboratory measurements are traceable to the primary RMs and, hence, to the international isotope reporting scales. Each stage of calibration will necessarily introduce additional uncertainty into the final result but it is possible to take steps to minimize the overall uncertainty:

- select RMs bracketing the range of possible δ-values expected;
- select RMs characterized by low uncertainty; and
- analyze RMs multiple times.

The laboratory must also periodically analyze primary and/or secondary RMs as a form of in-house proficiency test to ensure that the calibrations obtained from in-house materials are both accurate and repeatable.

1.3 Isotope ratio measurements

1.3.1 Data normalization

The aim of traceability is to ensure inter-laboratory comparability. To achieve this measured data must be *normalized* (sometimes described as *stretched* and *shifted*) to the appropriate international δ-scale. All of the numerical values that follow are taken from the CIAAW website (http://ciaaw.org, accessed December 2015).

1.3.2 Hydrogen measurements

The process of normalization for hydrogen isotopic measurements is illustrated in Figure 1.1 based on the 1993 recommendation of the CIAAW (Coplen 1994):

> "δ^2H values of all hydrogen-bearing materials be measured and expressed relative to VSMOW reference water on a scale normalized by assigning consensus values of −428‰ to SLAP reference water, and authors should clearly state so in their reports."

As noted above the supplies of both VSMOW and SLAP have been exhausted and replaced by two closely matched water samples christened VSMOW2 and SLAP2. These materials are ideal for the measurement of water samples as the isotopic compositions are widely spaced and encompass virtually all natural waters, with the possible exception of

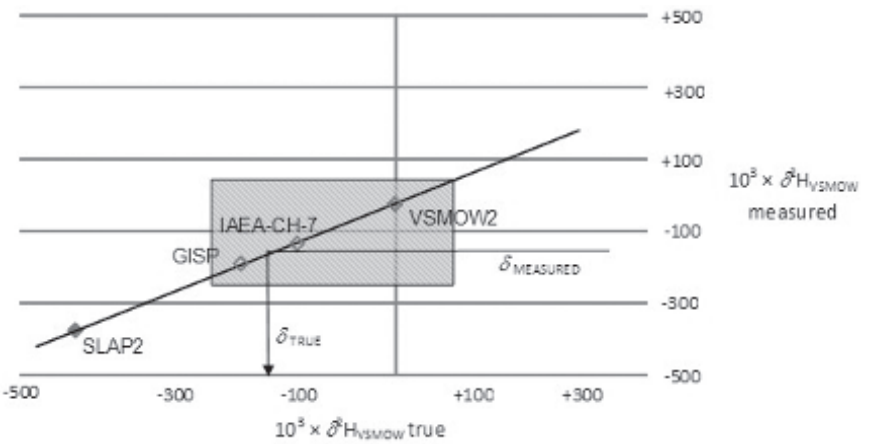

Figure 1.1 Normalization of δ^2H data to the internationally accepted reference materials VSMOW2 and SLAP2. GISP water or IAEA-CH-7 polyethylene can be used as a third calibration point and/or act as QC material. The shaded area shows the typical δ^2H composition of organic materials.

some tropical waters. An additional reference water, Greenland Ice Sheet Precipitation (GISP) has an isotopic composition conveniently between VSMOW2 and SLAP2 and can serve as a third calibration point and/or a QC material. A simple plot of the consensus *vs*. measured isotopic composition (Figure 1.1) then provides the means to normalize measured data to the international hydrogen δ-scale.

Measurements of hydrogen isotopic composition have become increasingly important in determining CoO and although VSMOW2 and SLAP2 are ideal RMs for the measurement of water samples, they are less than ideal for the measurement of solid organic materials. First, liquid samples are not readily compatible with the auto-samplers and inlet systems used to analyze solid materials. Second, the isotopic compositions of the reference waters do not extend to the more enriched values of some organic materials. The shaded area in Figure 1.1 shows the typical range of δ^2H compositions of organic materials.

Going some way to solve this problem, the United States Geological Survey (USGS) now supplies water RMs sealed in silver capsules (typically 0.15 or 0.25 µL) to facilitate introduction via a normal auto-sampler (Qi et al. 2010). A number of solid organic materials are also available (e.g., polyethylene foil, oil, and hair) with δ^2H values ranging from approximately −120 to −50‰. These materials provide options for additional calibration points and/or QC materials. The latter point is important to ensure that the inlet behaves the same towards silver encapsulated water and organic materials. Any off-set from the calibration line in Figure 1.1

will suggest that the inlet system does not behave the same towards water and organic samples.

RM suppliers are constantly working to develop new and replacement RMs to meet the increasing range of applications of stable isotope analysis techniques. The reader is strongly recommended to visit the websites of IAEA and USGS to check the availability of reference materials and the currently accepted δ-values and uncertainties.

1.3.3 Oxygen measurements

The normalization of oxygen measurements is very much akin to hydrogen with a CIAAW recommendation (Coplen 1994):

> "δ^{18}O values of all oxygen-bearing materials be expressed relative to VSMOW such that $\delta^{18}O_{SLAP/VSMOW} = -55.5‰$."

The normalization principles shown in Figure 1.1 also apply to δ^{18}O; both liquid and silver encapsulated SLAP2, GISP, and VSMOW2 provide the primary anchors with a range of solid materials available for calibration and QC. A number of solid RMs have significantly enriched isotopic compositions, which provide a convenient means to extend the normalization to positive δ^{18}O values—e.g., benzoic acids IAEA-601 (+23.1‰) and IAEA-602 (+71.3‰).

Although it is unlikely to be relevant to the field of food forensics, it should be noted that separate reporting scales exist for carbonates ($\delta^{18}O_{VPDB}$) and gaseous oxygen ($\delta^{18}O_{AIR\text{-}O2}$). The relationships between these scales are well established but not permanently defined and so, for simplicity, the isotopic compositions of oxygen bearing materials should be reported relative to VSMOW.

1.3.4 Carbon measurements

As noted above, the VPDB scale has been defined relative to NBS-19 limestone. To improve both the accuracy and precision of measurements, in 2005 the CIAAW recommended that (Coplen et al. 2006):

> "δ^{13}C values of all carbon-bearing materials be measured and expressed relative to the VPDB on a scale normalized by assigning consensus values of –46.6‰ to LSVEC lithium carbonate and +1.95‰ to NBS 19 calcium carbonate, and authors should clearly state so in their reports."

The principle of normalization for carbon isotopic compositions is, therefore, the same as applied to hydrogen and oxygen measurements. When the $\delta^{13}C_{VPDB}$ scale was redefined by reference to NBS-19 and LSVEC, both the assigned values and associated uncertainties of many of the

secondary reference materials were changed (Coplen et al. 2006). In general, this was a good thing, as uncertainties of many RMs improved; however, when comparing measurements reported prior to 2005, it may be necessary to re-scale the data.

In some respects NBS-19 and LSVEC are ideal RMs for the normalization of carbon isotopic ratios as they have widely different isotopic compositions, which span the likely composition range for organic compounds (Figure 1.2). Unfortunately, these RMs also come with a number of limitations. First, these materials are not readily available—supplies were always restricted, for conservation, but both NBS-19 and LSVEC are currently *out-of-stock* and no replacements are currently available. Second, the VPDB scale is defined by the carbon dioxide liberated from NBS-19 limestone by treatment with phosphoric acid. In contrast, the most common IRMS sample preparation/inlet device is an Elemental Analyzer (EA) in which a sample is combusted in the presence of oxygen; trying to convert $[CO_3]^{2-}$ to CO_2 in an oxidizing environment is not a favorable reaction.

To overcome the availability and/or suitability for the primary RMs, a laboratory has a number of options:

- normalize measured data using secondary RMs [e.g., USGS-40 ($\delta^{13}C$ = −26.39‰) and USGS-41 ($\delta^{13}C$ = +37.6‰)]
- prepare in-house RMs to closely match the isotopic composition of the primary RMs (Carter and Fry 2013b).

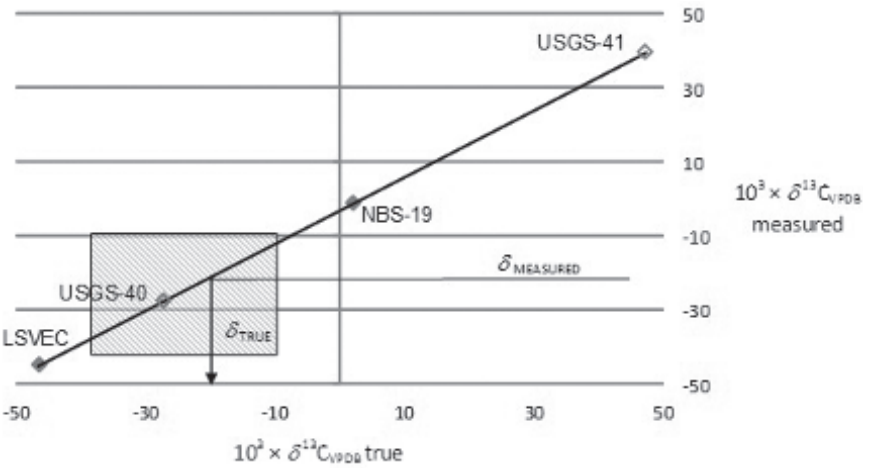

Figure 1.2 Normalization of $\delta^{13}C$ data to the internationally accepted reference materials NBS-19 and LSVEC. USGS-40 and USGS-41 provide alternative calibration points and/or act as QC material. The shaded area shows the typical $\delta^{13}C$ composition of organic materials.

The plus point of both these options is replacing carbonates with organic compounds, which can be a close matrix match for typical laboratory samples and will be far more readily converted to CO_2 during combustion in an EA. As a negative point, the overall uncertainty budget for measurements must increase due to the additional links in the chain of calibration; however, with careful selection of RMs, this increase in uncertainty can be kept to a minimum.

1.3.5 Nitrogen and sulfur measurements

Both the $\delta^{15}N_{AIR}$ and $\delta^{34}S_{VCDT}$ scales are still defined relative to a single primary RM in a similar manner to the original definitions of VPDB:

a material with $\delta^{15}N = 0‰$ relative to N_{AIR}

$^{14}N = 0.996\ 337(4)$
$^{15}N = 0.003\ 663(4)$

IAEA-S-1 silver sulfide = –0.3‰ exactly.

No doubt, at some time, these scales will be formally defined by reference to two primary RMs. In the interim, a number of secondary RMs exist that meet some of the basic requirements for RMs including widely spaced δ-values, bracketing likely sample values. Some examples are given in Table 1.3.

Table 1.3 Secondary reference materials suitable for the normalization of nitrogen and sulfur stable isotope measurements.

Material	Nature	Value (‰)	Unit	SD
USGS25	ammonium sulfate	–30.3	$\delta^{15}N_{AIR}$	0.4
IAEA-N-1	ammonium sulfate	+0.4	$\delta^{15}N_{AIR}$	0.2
IAEA-N-2	ammonium sulfate	+20.3	$\delta^{15}N_{AIR}$	0.2
IAEA-S-2	silver sulfide	+22.6	$\delta^{34}S_{VCDT}$	0.16
IAEA-S-3	silver sulfide	–32.5	$\delta^{34}S_{VCDT}$	0.16

1.4 Good practice

1.4.1 Good practice in practice

The Forensic Isotope Ratio Mass Spectrometry (FIRMS) network, in conjunction with the UK National Measurement facility, has produced an excellent Good Practice Guide (GPG) to stable isotope measurements. This is available to download for free (forensic-isotopes.org/assets/IRMS%20Guide%20Finalv3.1_Web.pdf) and the reader is strongly encouraged to obtain a copy and to absorb its contents.

Always remember that the need for good practice and quality control in food forensics is not limited to stable isotope measurements and must extend to all physical and chemical measurements that are applied. It is not the intention of this chapter to repeat the advice of the FIRMS' GPG but simply provide a summary of what is necessary for a laboratory to obtain data that are:

- repeatable over a long time period within the same laboratory
- comparable to data from other laboratories

Typically, food forensics is a comparative technique and the strength of evidence that can be presented is very much dependent on the size (and quality) of any background database. Both of the requirements above must be met if data are to be reliable in building reference datasets (or *databases*).

The best way to achieve reliable data can be summarized in two points:

- the Principle of Identical Treatment (PIT)
- traceability to the international δ-scales

The PIT simply requires that samples and reference materials are treated in an identical manner and should be applied to all physical and chemical treatments of the sample. Also (and most importantly) the inlet of the instrument (typically an EA and open-split arrangement) must behave in an identical manner to both samples and standards. In general this requires that the elements in a sample are converted quantitatively to CO_2 and N_2 (for measurement of carbon and nitrogen isotope abundances, respectively), H_2 and CO (for measurement of hydrogen and oxygen isotope abundances, respectively), or SO_2 (for measurement of sulfur isotope abundances) and that these gases are transported to the ion source of the instrument without isotopic fractionation (or at least with consistent fractionation).

Once samples have been prepared and measured according to the PIT it is important to ensure that identical treatment is applied to all subsequent processing of the data; sometimes described as the Principle of Identical Correction (PIC) (Carter and Fry 2013a).

1.4.2 On-board (hidden) corrections

Instrument software has become increasingly sophisticated and allows an analyst to apply a number of corrections that were previously performed in external spreadsheets or Laboratory Information Management Systems (LIMS). Most modern IRMS instrument software will automatically apply a number of corrections to the measured data before presenting results to the analyst. Many of these are discussed in the FIRMS' GPG, but in summary, data will be "normalized" to a single calibration point (often a working gas) (Paul et al. 2007) and, for certain gases, a correction is

applied for $^{16}O/^{17}O/^{18}O$ ratios (Brand et al. 2010). The software may give an analyst various options for these corrections but so long as they are applied evenhandedly to samples and reference materials, results should be consistent.

1.4.3 Optional corrections

In addition to instrument-based corrections and the essential normalization to international δ-scales, a number of other corrections are sometimes applied to isotope ratio measurement results. When establishing analytical protocols a laboratory must determine whether these corrections are necessary or useful. Applying too many "corrections" to data can introduce as many problems as are solved. As a heuristic technique, a laboratory should only apply corrections that have a significant and positive effect or they simply become *fudge-factors*!

1.4.4 Blank correction

The purpose of a *blank correction* is to subtract the contribution of any gases evolved during a blank measurement (i.e., an empty tin or silver capsule) and for a number of reasons this correction is best avoided. *Blank correction* only becomes important when sample size is limited or the abundance of a certain element in the matrix is very low. Typically in food forensics sample sizes are large, given that only a few hundred micrograms of any element is required for an analysis. Homogeneity is much more of a problem as discussed in Chapter 2.

In addition, the apparent isotopic composition of small peaks can vary wildly with varying integration parameters and determining the true isotopic composition of an instrument blank is not as simple as measuring the response from a few empty capsules and subtracting the value. The true isotopic composition of the blank must be determined by analyzing a series of dilutions for two reference materials with different isotopic compositions (Carter and Fry 2013a).

A much more robust practice is to establish the acceptable size of a blank analysis; if a blank is less than 1% of a normal sample peak height, it can have little effect on the measured isotopic composition. Once suitable acceptance criteria have been established, it is important to measure the size of the blank before any sample measurements. A high blank is indicative of contamination—capsules, forceps, preparation area, etc. When a high blank is identified the important action is to identify and address the cause and not simply correct for it.

1.4.5 Size correction

Modern IRMS instruments have detectors and amplifiers with excellent linearity and very wide dynamic range. Unfortunately, because the ion source of the instrument is tightly enclosed, to maximize sensitivity, the apparent isotopic composition of a sample may vary dependent on the amount of gas entering the ion source (i.e., the peak size) as shown in Table 1.4. The linearity of an IRMS instrument can be measured on a daily basis either by introducing samples or working gas pulses of varying size as illustrated in Figure 1.3. When validating a measurement protocol it is important to measure linearity by both means and, hopefully, obtain the same correction factor.

The linearity is simply the slope of the data (δ-value *vs*. amplitude); in this example the slope is 0.02‰ per 1,000 mV. This may seem a very small

Table 1.4 An example of linearity measurement data of an IRMS instrument.

Signal amplitude (mV)	δ-value (‰)
1078	−0.109
4797	0
6537	+0.040
11181	+0.102
15185	+0.189
22273	+0.344

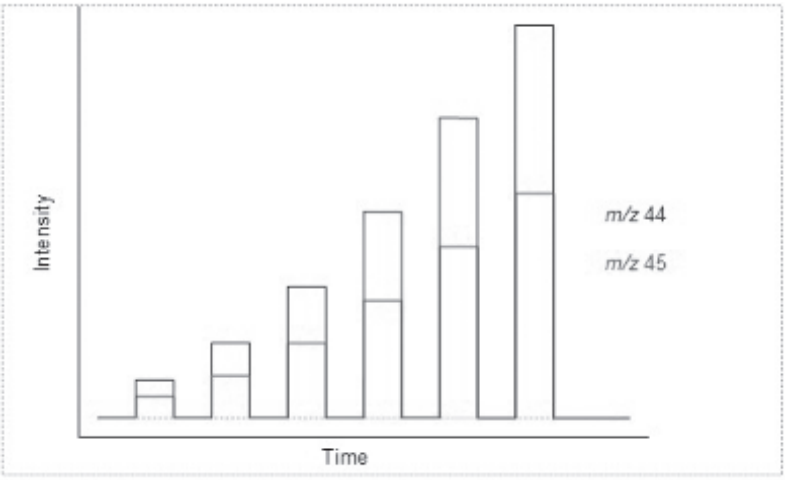

Figure 1.3 An example of linearity measurement data display of an IRMS instrument.

correction but, if sample peaks vary from 5,000 to 15,000 mV the correction would amount to 0.2‰, which is larger than typical experimental error for $\delta^{13}C$ determination. If the linearity correction is small it can simply be ignored but, like blank correction, it is important to *measure and monitor* the linearity. A gradual trend or sharp change in linearity may point to a problem with the IRMS instrument or inlet systems.

1.4.6 Drift correction

Despite the high stability of modern instrument electronics, the δ-scale of an IRMS will inevitably *drift* over time most often due to changes in the isotopic composition of background gases within the ion source. The phenomenon is often most pronounced when measuring hydrogen isotopes as this gas can diffuse against the flow of the vacuum pumps back into the ion source. Drifts in the δ-scale are readily identified through the regular analysis of in-house QC materials. To be useful, such a material must be analyzed every 5–10 samples depending on the number of replicates for each sample.

A number of publications describe how to adopt an effective QC regime (e.g., Brand 2009; Coplen and Qi 2009) typically based on control charts or Shewhart charts with warning (the accepted value ± 2 standard deviations) and action limits (the accepted value ± 3 standard deviations).

A good analytical protocol (and a good analyst) will define appropriate actions once the defined control limit for QC is breached:

- stop the analytical sequence,
- re-test the QC material to ensure that the breach was not an outlier, and/or
- re-test all analytical samples following the last successful QC.

The trade-off in this process is that if you run a QC material every 5 samples you will analyze more QCs but you risk only 5 sample results; if you run a QC material every 10 samples you analyze fewer QCs but you risk 10 sample results.

An alternative approach is *drift correction*, which uses the QC results as a continuously variable offset for the analytical data. This approach relies on two assumptions:

- the instrument drift has an equal effect on QC material and samples, and
- the instrument drift is linear between QC material measurements.

If these assumptions are considered to be correct, the process is easily implemented via instrument software or external spreadsheets. When adopting this approach it is sensible however, to run a second QC material between drift-correction RMs to ensure that the drift correction is working effectively, otherwise this becomes a *fudge-factor*.

1.4.7 Memory correction

For certain measurements, notably high temperature reduction over glassy carbon, the measured isotopic composition of a sample can be affected by the composition of the previous samples. For example, six replicate measurements of SLAP2 (δ^2H = −427.5‰) followed by six replicate measurements of VSMOW2 (δ^2H = 0.0‰) might well show a pattern:

−428.0	−428.1	−427.8	−428.0	−428.3	−428.1
−12.2	−4.0	−1.9	−0.4	−0.3	−0.3

In this example, the δ-values measured for VSMOW2 exhibit a memory effect from the SLAP2 previously analyzed, which contains far less ^{2}H relative to VSMOW2.

Researchers have presented algorithms that correct the measured δ-value of a sample by subtracting some proportion of the measured value of up to three previous samples (e.g., Gröning 2011). These algorithms are typically applied to water samples in which the peak size is relatively constant; for solid samples in which the peak size can be variable, an algorithm would also need to account for the size of preceding samples.

A simpler and more robust solution to overcome memory effect is to ignore the first few replicate analyses. The disadvantage of this approach is that it is wasteful of analytical time especially for the δ^2H or δ^{18}O analysis of solid samples for which the silver residue must be emptied every one hundred to two hundred samples.

1.4.8 Summing up

In order for stable isotope ratio measurements to be useful as a tool in food forensics they must be both repeatable (within-laboratory) and traceable to international reporting scales (between-laboratory). To achieve results that are fit-for-purpose the following steps are recommended (Carter and Fry 2013a):

1. obtain RMs with internationally agreed δ-values (spanning the likely range of δ-values for samples),
2. prepare matrix matched in-house RMs (calibrate these against the international RMs and calculate the overall uncertainty),
3. use RMs to determine which corrections are necessary or desirable (linearity, blank, drift, memory),
4. use RMs to demonstrate adherence to the Principle of Identical Treatment of sample preparation and inlet systems (focus on the difference, Δ, between RMs),
5. prepare in-house QC materials (chemically similar to routine samples and with typical δ-values),

6. develop an analytical protocol that includes the routine analysis of QC materials (with every batch of samples!),
7. ensure that sample and QC data are processed and corrected in an identical manner (by both instrument and external software),
8. monitor the δ-values of QC materials and act on any deviations or trends, and
9. periodically analyze international RMs as an in-house proficiency test (act on any significant deviations or trends).

1.5 Nomenclature

When reporting stable isotope ratio measurements it is important to conform to a standard nomenclature:

- δ and Δ should be presented in italic font
- δ-values should be preceded by a + or − symbol

the subscript text can have a subtle but important meaning:

Example	
δ^2H	A general form of reporting isotopic difference
δ^2H_{VSMOW}	The data are traceable to the international reporting scale
$\delta^2H_{VSMOW\text{-}SLAP}$	The data are traceable to the international reporting scale through two point normalization using the accepted isotopic compositions of the specified RMs.

δ-values are typically presented as ± the standard uncertainty at one sigma level, which can be derived from the standard deviation of replicate measurements or from the complete uncertainty budget, which should be defined. When large tables of data are presented, it can be acceptable simply to report the median uncertainty for measurements of a given element.

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5 Chapter5. Data Analysis InterpretationForensic Applications and Examples

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Figure 10.6 Three dimensional plot of the reference MFA samples (dots) and the case samples

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