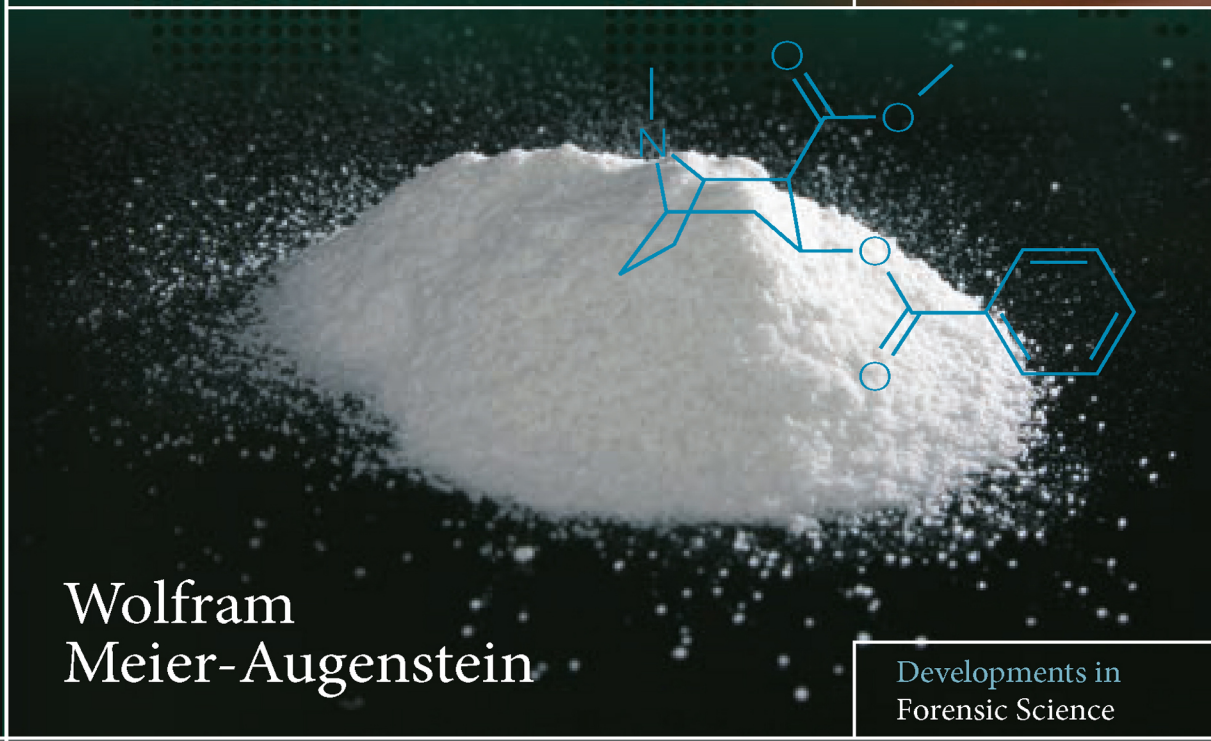
2ND EDITION

STABLE ISOTOPE FORENSICS

Methods and Forensic Applications of Stable Isotope Analysis



Wolfram Meier-Augenstein

Developments in Forensic Science

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Stable Isotope Forensics

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Methods and Forensic Applications of Stable Isotope Analysis

Second Edition

Wolfram Meier-Augenstein

Robert Gordon University
Aberdeen, UK

WILEY

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Series Foreword

The world of forensic science is changing at a very fast pace in terms of the provision of forensic science services, the development of technologies and knowledge and the interpretation of analytical and other data as it is applied within forensic practice. Practicing forensic scientists are constantly striving to deliver the very best for the judicial process and as such need a reliable and robust knowledge base within their diverse disciplines. It is hoped that this book series will provide a resource by which such knowledge can be underpinned for both students and practitioners of forensic science alike.

It is the objective of this book series to provide a valuable resource for forensic science practitioners, educators and others in that regard. The books developed and published within this series come from some of the leading researchers and practitioners in their fields and will provide essential and relevant information to the reader.

Professor Niamh NicDaéid
Series Editor

Foreword by Dame Sue Black

I am so delighted to be asked to write the foreword for a text where I understand so little of the background science. A reasonable question might be, then why ask a forensic anthropologist to do this when she barely passed Higher Chemistry and has absolutely no experience in the field of stable isotope analysis? One of the most important aspects of working within a forensic team is to know the limits of one's own ability and to recognize and utilize the strengths of others. It has been my pleasure to work with Wolfram for many years and when forensic casework comes to me, it is without second thought that I pass it on to him knowing that the investigative authorities will not be hoodwinked by a pseudoscientist.

A single-author text in these days is rare and the value of this book lies in the dedication and experience of the author, which is evident in the clarity of prose, the honest illustration of evidence and the realistic practical application of the subject – it makes this a text of genuine scientific value. That a second edition has been requested is a clear indication that the field is still progressing and that new research is still being reported. In the current world of forensic science flux, it is vital that robust scientific research endeavours continue and that it be reported not only in published peer-reviewed papers but collocated in scholarly tomes for easy reference.

In my early discussions with Wolfram I admit to having been a bit of a sceptic, but over time I have been educated and fully persuaded of the value of stable isotope analysis to the world of provenancing and human identification. There have been several instances where conclusions drawn regarding ethnic origin based on forensic anthropological examination of skeletal remains were corroborated independently by results from stable isotope analysis of bone and teeth. One need only read the case histories included in the text to appreciate the practical value of this approach to forensic investigations and, in particular, when attempting to establish the identity of the deceased, which is a pivotal component of any successful investigation.

For me, one of the most important and reassuring aspects of this text is its brutal openness, honesty and transparency. Without apology it identifies equally where are the strengths and limitations of the science and its interpretation for forensic purposes. Whilst this book will challenge those who are not chemically literate, it will quickly become established as the "go-to" text for all practitioners and end users

who require to have a firm grasp of the complexities of the subject if its relevance is to be fully understood as a part of intelligence-based investigation.

Prof. Dame Sue Black, PhD, DBE, OBE, FRSE
Leverhulme Research Centre for Forensic Science
University of Dundee, UK

Foreword by Commissioner Mark Harrison

As an early adopter in applying stable isotope forensic techniques to aid my own criminal investigations, I have followed closely its further development and I am honoured to write the foreword for this second edition of *Stable Isotope Forensics* by Dr Meier-Augenstein.

Since Dr Meier-Augenstein's first edition of this textbook, crime, through globalization has become more transnational requiring law enforcement to operate in a criminal environment where uncertainty and complexity are increasing and their time to respond is decreasing. Forensic science's response to these challenges has seen the expansion of its contribution beyond prosecution and more to aid investigators in the disruption of crime.

This changing emphasis by law enforcement to one of disrupting organized crime and terrorism is enabling stable isotope forensics (SIF) to increase its value to investigators whereby its contribution to casework is both evidential and in the provision of forensic intelligence.

Criminal syndicates are increasingly interconnected and often commodity based and it is here that SIF profiling is adding value in diverse areas such as narcotics, human trafficking and environmental crime, where provenance is an investigative priority enabling the mapping of criminal networks and the source and transit countries they use. Further SIF innovation has been seen in recent times through countries testing the waste water in their cities and towns to gain greater understanding of the geographic and demographic profiles of drug use, bringing together law enforcement and health agencies in harm reduction programs.

Since the first edition of this book, terrorist groups have become less formalized and radicalization affects all societies through the interconnected world of the Internet. The online-inspired foreign fighter phenomenon has enabled opportunities for SIF profiles to provide significant forensic intelligence value to law enforcement in provenancing the origin of these terrorists and their movements throughout the world.

The scope of SIF contribution is expanding and is only currently limited due to the provision of reference databases. The next decade will see increasing convergences with other techniques such as DNA phenotyping to provide a more holistic picture of criminal identity. Big data challenges will also be addressed to

enable timely processing of samples for both evidentiary and forensic intelligence purposes to rapidly answer the who, what, where and how that are, and will continue to be the key drivers of all criminal investigations.

Commissioner Mark Harrison, MBE
Head of Criminal Intelligence, Australian Federal Police

Foreword to the 1st Edition

I am delighted to be able to write the foreword for this, the first textbook on stable isotope forensics.

The coverage is wide, ranging from fundamentals to policy issues, and therefore this text will be of benefit to practitioners, researchers and investigators, indeed to anyone who has an interest in this new forensic discipline.

The year 2001 saw the formation of the Forensic Isotope Ratio Mass Spectrometry (FIRMS) Network. Since then much has been achieved in terms of advancing the forensic application of stable isotope analysis, this textbook being the latest significant step.

These advances have been made in the face of considerable challenges resulting from the novelty and complexity of the technique. Isotope forensics has already proved a powerful tool in the investigation and prosecution of high-profile crimes, including terrorism. Stable isotope analysis enables questions regarding the source and history of illicit and other forensic materials to be addressed – questions that might otherwise remain unanswered.

Isotope forensics is now being widely adopted for profiling illicit materials and human provenancing. Stable isotope analysis has already been used successfully in two major terrorist trials in the United Kingdom, and in a variety of investigations and trials in the United Kingdom, Europe and the United States.

Dr Meier-Augenstein is to be commended for his vision in recognizing the forensic potential of stable isotopes, for his energy in developing and optimizing the methodology, and in promoting the technique to end users. He is also well aware of the risk of contributing to a miscarriage of justice and recognizes that only an appropriate regulatory framework can significantly mitigate that risk.

The development of suitable databases of reference materials and appropriate tools for evaluation remain significant tasks; once complete the next decade should see isotope forensics taking a deserved place in mainstream forensic science and, to a greater extent, contributing to the efficient and effective delivery of justice.

Sean Doyle
Past Chair of the FIRMS Network
Principal Scientist, Forensic Explosives Laboratory,
Defence Science and Technology Laboratory
September 2009

Book Endorsements

"All students of forensic criminology, and all law enforcement officers responsible for the investigation of serious crime, will want to study this book. Wolfram highlights the value, and future potential, of stable isotope forensics as an emerging powerful tool in the investigation of crime."

Roy McComb, Deputy Director, Specialist Investigations, National Crime Agency (NCA), UK

"This is an important and timely book. Having employed the technique in a murder investigation, I can say that this book is a must-read for police officers, forensic scientists and others who want to learn about the applied implications of Stable Isotope Forensics."

Inspector John C. House, M.O.M., M.Sc., Royal Newfoundland Constabulary, Canada

"Stable Isotope Forensics is an important major work and in my opinion will have a significant impact."

Sean Doyle, Past Chair of the FIRMS network and Principal Scientist, Forensic Explosives Laboratory, Dstl

Preface to the 2nd Edition

When John Wiley & Sons approached me asking how I would feel about preparing a second edition of this book I was flattered and pleasantly surprised in equal measure. I never expected this book to be so successful as to merit a second edition. However, here we are seven years after publication of the first edition with me writing a personal foreword I never expected to write. Even though I had told myself never to repeat the experience of writing a book I found myself saying yes for chiefly two reasons. On the upside, since 2010 a growing body of casework and research in the subject areas of this book has increased our understanding of stable isotope forensics, its potential as well as its limitations. On the downside, it has to be said also that many a publication or case report has come to light reporting "forensic" stable isotope data claiming to identify or authenticate a source or origin of a given compound or material but based on analytical methods which upon critical inspection, to quote an esteemed colleague of mine, are nothing but "unmitigated s###".

More often than not, the main reason for this devastating assessment is the lack of properly validated data that are neither traceable to internationally recognized scale reference materials nor internationally comparable and, hence, not reproducible. In almost all instances of this kind, the reported stable isotope abundance values were the result either of the indefensible practice of one-point "calibration" or, worse, of a mere comparison against an untraceable stable isotope abundance value of a self-prepared standard or a cylinder gas. To quote another, equally esteemed, colleague, justifying such "indefensible calibration practices" is like following or promoting so-called toilet wall wisdom (*Scheißhausparolen*) as the gospel's truth.

I appreciate the above quotes are strong language and may be considered offensive by some. However, I chose to quote these comments because this book's aim is to provide information not only for practitioners and students of forensic science but also for end users such as law enforcement officers and legal professionals. To my mind it is therefore important to present both the potential and the pitfalls of forensic stable isotope analysis and to provide examples for both. For example, failure to comply with internationally accepted guidelines for stable isotope ratio measurement and reporting results thereof and as a consequence reporting stable isotope abundance values that cannot be repeated, reproduced or compared on a like-for-like basis undermines not just confidence in the data but confidence in the conclusions drawn. This would be particularly regrettable if such data were to be presented and refuted in court resulting either in charges against a guilty

person being dismissed or an innocent person being convicted for a crime he or she did not commit. Another outcome would be flawed intelligence based on not-fit-for-purpose stable isotope data resulting in investigative efforts and valuable police man-hours being wasted. Presenting flawed data and thus flawed conclusions in this arena will ultimately destroy the confidence and respect the stable isotope community currently enjoys, not to mention the knock-on effects this will have on any future use of stable isotope abundance data as a forensic intelligence tool or indeed as evidence presented in court. In this context it does not matter if flawed stable isotope abundance data reported, even published, in well-respected journals on forensic science or legal medicine have been generated with good intentions. As the saying goes, the road to hell is paved with good intentions. However, not making the efforts necessary to generate traceable, reproducible and thus comparable stable isotope data "because it's difficult" is unforgiveable. That being said, I would like to stress any references quoted in this book to exemplify inappropriate methodology and not-fit-for-(forensic)-purpose data are just that, examples. Any criticism with regards to methodology notwithstanding, this is not meant to distract from the effort and time otherwise invested in the work reported in such references or from the potential of the observations made to gain new insights or to serve as springboard for future research.

I therefore intend the second edition of this book also to be an appeal to students, scientists, practitioners, crime scene officers, police officers and lawyers acting for the prosecution or the defence to be extra vigilant. I urge you to critically read publications and case reports reporting or relying on stable isotope abundance data obtained by continuous flow – isotope ratio mass spectrometry and to thoroughly check for compliance with international guidelines as well as the principles of analysis referred to and described in this book. Unfortunately, it is inevitable that methodological flaws and mistakes that have slipped through the net of peer review once will be perpetuated by others.

Consequently, this second edition is more than an updated version of the first edition focusing on more than just on new case examples and the latest findings from original research. In order to address the problems arising from non-comparable and therefore unfit-for-purpose stable isotope data, Part II of the book has all but been rewritten with great emphasis on important key aspects of stable isotope analysis in general and forensic stable isotope analysis in particular. The attention of practitioners, would-be practitioners and end users is therefore drawn to the chapters dealing with the various aspects of quality control and quality assurance such as scale normalization (i.e. isotopic calibration), the identical treatment principle, hydrogen exchange and accreditation.

But of course, this second edition does also include exciting new ideas and key findings from original research in stable isotope forensics carried out during the last six years. It also includes further case examples, covering a spectrum from the fascinating to the heartbreaking.

As in the first edition I have adopted two styles of writing; the style typically used when writing articles for publication in scientific journals and a personal style narrating in the first person. I have used the latter to clearly identify passages in which I report a personal experience, relay a personal impression or observation, or state a personal opinion. Any mistakes or misconceptions expressed therein are my own.

Wolfram Meier-Augenstein
June 2017

List of Abbreviations

AAFS	American Academy of Forensic Sciences
ABFA	American Board of Forensic Anthropology
AFP	Australian Federal Police
BKA	Bundeskriminalamt (Germany)
BSIA	Bulk Stable Isotope Analysis
C	Combustion, or, more generally, Conversion
CAHId	Centre for Anatomy and Human Identification
CIAAW	Commission on Isotopic Abundances and Atomic Weights
CF-IRMS	Continuous Flow - Isotope Ratio Mass Spectrometry
CM	Calibration Materials
CSIA	Compound Specific Isotope Analysis
DEA	Drug Enforcement Agency (US)
EA	Elemental Analyser
ENFSI	European Network of Forensic Science Institutes
FBI	Federal Bureau of Investigation
FC	Faraday Cup
FEL	Forensic Explosives Laboratory
FID	Flame Ionisation Detector
FIRMS	Forensic Isotope Ratio Mass Spectrometry network
GC	Gas Chromatography
GC/MS	Gas Chromatography coupled to a Mass Spectrometer (or Gas Chromatography/Mass Spectrometry)
GISP	Greenland Ice Sheet Precipitation
GMWL	Global Meteoric Water Line
HCA	Hierarchical Cluster Analysis
HPLC	High Performance Liquid Chromatography
HTC/EA	High Temperature Conversion/Elemental Analyser
IAEA	International Atomic Energy Agency
IRMS	Isotope Ratio Mass Spectrometry (or Spectrometer)
IUPAC	International Union of Pure and Applied Chemistry
LC	Liquid Chromatography
LR	Likelihood Ratio
MC	Multi-Collector
MS	Mass Spectrometry (or Mass Spectrometer)

NCA	National Crime Agency (UK)
NIST	National Institute of Standards and Technology (US)
PCA	Principal Component Analysis
Py-GC/MS	pyrolysis-GC/MS
PVC	Polyvinylchloride
RM	Reference Material
SIA	Stable Isotope Analysis
SLAP	Standard Light Antarctic Precipitation
SOCA	Serious Organised Crime Agency (UK)
TCD	Thermal Conductivity Detector
UNPER	Unidentified (Unknown) Person
USGS	United States Geological Survey
VCDT	Vienna Cañon Diablo Troilite
VPDB	Vienna Pee Dee Belemnite
VSMOW	Vienna Standard Mean Ocean Water

About the Companion Website

Don’t forget to visit the companion website for this book:

www.wiley.com/go/meier_augenstein/sif

There you will find valuable material designed to enhance your learning, including:

Fully Worked Out Solutions to Posed Test Questions

- Reference List of Example Publications for Chapter II.3
- Reference List of Example Publications for Chapter II.4
- Reference List of Example Publications for Chapter III.1
- Reference List of Example Publications for Chapter III.7
- All Questions and Answers Provided for Website

Introduction

Stable Isotope 'Profiling' or Chemical 'DNA': A New Dawn for Forensic Chemistry?

Starting with the conclusion first, I would say neither of the above two terms is appropriate, although I am convinced information locked into the stable isotopic composition of physical evidence may well represent a new dawn for forensic chemistry.

The title for this general introduction was chosen deliberately as an analogy for the term "DNA fingerprinting," coined by Professor Sir Alec J. Jeffreys, to draw the reader's attention to the remarkable analogy between the organic, life-defining material DNA and the more basic, and on their own lifeless, chemical elements in their various isotopic forms when examined in the context of forensic sciences in general and human provenancing in particular. It is also my intention to alert readers from the start to the dangers of expecting miracles of stable isotope forensics. DNA evidence is at its most powerful when it can be matched against a comparative sample or a database entry, and the same is true to a degree for the information locked into the isotopic composition of a given material. Similarly, the random match probability of 1 in 1 billion for a DNA match based on 10 loci and the theoretical match probability of an accidental false positive match of a multi-isotope signature are also seemingly matched, with a multivariate or multifactor probabilistic equation being the common denominator for both. If we consider a material such as hair keratin and we make the simplifying assumption this material may exist naturally in as many different isotopic states per element as there are whole numbers in the natural abundance range for each stable isotope on the δ-scale (Fry, 2006), we can calculate a hypothetical figure for the accidental match probability of such a multi-element isotope analysis that is comparable to that of a DNA fingerprint.

For example, the widest possible natural abundance range for carbon-13 is 110 ‰ (Fry, 2006) so for the purpose of this example we could say keratin can assume 110 different integer carbon-13 values. Analysing hair keratin for its isotopic composition with regard to the light elements hydrogen (H), carbon (C), nitrogen (N), oxygen (O) and sulfur (S) could thus theoretically yield a combined specificity ranging from 1 in 638 million to 1 in 103.95 billion. In fact, one can calculate that the analysis of hair keratin for its isotopic composition with regards

to H, C, N and S would theoretically yield a combined specificity of 1 in 1 billion, thus suggesting a "stable isotope profile" or "stable isotope signature" based on these four letters of the chemical alphabet having the same accidental match probability as a DNA fingerprint that ultimately is based on the four letters of the DNA alphabet, A (adenine), C (cytosine), G (guanine) and T (thymine) (see Box). However, it has to be stressed that it has as yet not been fully explored if this hypothetical level of random match probability, and hence level of discrimination, is actually achievable given that the natural abundance ranges in which compounds or materials can occur are usually much narrower than the widest possible theoretical range. We will learn more about this in the course this book. Forensic scientists and statisticians such as Jurian Hoogewerff (University of Canberra) and James Curran (University of Auckland) suggest more conservative estimates, putting the potentially realised random match probability of stable isotope signatures at levels between 1 in 10,000 and 1 in 1 million depending on the nature and history of the material under investigation. However, even at these levels stable isotope profiling is still a potentially powerful forensic tool.

Analogies between DNA and stable isotopes of light elements

Biological DNA versus Chemical "DNA"	
Alphabet of Biological DNA comprises the letters	Alphabet of Chemical "DNA" comprises the letters
A	^{2}H
C	^{13}C
G	^{15}N
D	^{18}O
[U]	[^{34}S]

The random match probability of Biological DNA is approximately 1 : 1 billion (1×10^{9}) for a DNA profile based on 10 loci.
The random match probability of a five-element stable isotope profile can theoretically range from 1 : 693 million (6.93×10^{8}) to as high as $1 : 1.04 \times 10^{11}$.
Note: This is for illustrative purposes only and does not denote any equivalence between DNA bases and chemical elements.

While one can make a good case that the isotopic abundances of ^{2}H, ^{13}C, ^{15}N and ^{34}S are independent variables and figures representing their abundance range can hence be combined in a probabilistic equation, the same is not entirely the case for ^{2}H and ^{18}O, which when originating from water may behave like dependent variables. More relevant to this issue is the question if and to what degree isotopic abundance varies for any given material or compound. While across all materials

and compounds known to man ^{13}C isotopic abundance may indeed stretch across a range of 110 δ-units, its range in a particular material, such as coca leaves, may only extend to 7 δ-units (Ehleringer *et al*., 2000).

Another reason why the analogy between DNA fingerprinting and stable isotope profiling should only be used in conjunction with qualifying statements is the fact that both a DNA fingerprint and a physical fingerprint are immutable, that is, they do not change over time. Drawing on an example from environmental forensics, calling a gas chromatography (GC) or gas chromatography-mass spectrometry (GC/MS) profile from a sample of crude oil spillage a fingerprint of that oil is a misnomer since ageing processes such as evaporation will lead to changes in the oil's composition with regard to the relative abundance of its individual constituents. Incidentally, due to isotopic fractionation during evaporation the isotopic composition of any residual oil compound will also have changed when compared to its isotopic composition at the point of origin. A more apt analogy would therefore be the use of the term stable isotope signature. Just as a person's signature can change over time or under the burden of stress, so the stable isotopic composition of the residual sample from a material susceptible to evaporative loss may have changed by the time it ends up in our laboratories. Furthermore, in the same way a forensic expert relies on more than one physicochemical characteristic as well as drawing on experience and contextual information to arrive at an interpretation regarding similarity or dissimilarity, the stable isotope scientist combines measured data with experience, expertise and contextual information to come to a conclusion as to what a given stable isotope signature does or does not reveal.

Despite these caveats it is easy to see why the prospect of having such powerful a tool at one's disposal for combating crime and terrorism has caused a lot of excitement in both the end-user and scientific communities. However, if the history of applying DNA fingerprinting in a forensic context has taught us anything then it is this: great potential is no substitute for good forensic science, and good forensic science cannot be rushed or packaged to meet externally driven agendas. At first there was no great interest in this new forensic technique, but after a few spectacular successes demand for what seemed to be the silver bullet to connect suspect perpetrators to victims or crime scenes increased faster than research still concerned with answering underlying fundamental questions could keep up with, and history has all but repeated itself recently on the subject of low template DNA. Good forensic science cannot be rushed but is the outcome of good forensic science teaching and research, which in turn become the foundation of good forensic practice. While the former requires proper funding the latter requires proper regulation, and both requirements must be addressed and met.

Not surprisingly, therefore, even at the time of writing the second edition of this book we still have a mountain to climb to turn stable isotope forensics into a properly validated forensic analytical tool or technique that is fit for purpose. Even though this technique has been successfully applied in a number of high-profile criminal cases where salient questions could be answered by comparative analysis,

this should not blind us to the fact that a considerable amount of time, effort, money and careful consideration still has to be spent to develop and finely hone this technique into the sharp investigative tool it promises to be.

Similar to DNA, data have to be generated and databases have to be compiled for a statistically meaningful underpinning of this technique and the interpretation of its analytical results. Equally important, if not more so, all the steps from sample collection, sample storage and sample preparation to analytical measurement and the final data reduction have to be carefully examined either to avoid process artefacts or, if unavoidable, to quantify such artefacts and develop fit-for-purpose correction protocols to avoid stable isotope forensics suffering the same fate as low template DNA.

One way of ensuring appropriate and well-advised use of this technique in a forensic context is to advise and instruct current and future generations of forensic scientists in this technique as early as possible. Fortunately this is possible in spite of the aforementioned drawbacks for two reasons: (i) thanks to end-user interest there is a sufficient number of actual case work and associated background research and their results provide part of the foundations on which this book is built; (ii) contrary to the misconception of many an analytical chemist there is a huge body of knowledge and insight gained in scientific areas, including archaeology, biochemistry, environmental chemistry, geochemistry, palaeo-ecology and zoology, to name but a few, that is based on stable isotope chemistry and stable isotope analytical techniques.

In this book, the theory, instrumentation, potential and pitfalls of stable isotope analytical techniques are discussed in such a way as to provide an appreciation and better understanding of this analytical technique. To this end some of the physical chemistry background relating to mass discrimination, isotopic fractionation and mass balance is only touched upon, while some of the practical consequences of the aforementioned on the analytical process, the kind of information obtainable, or the level of uncertainty associated with stable isotope data from a particular type of sample are discussed in finer detail. There are a number of excellent books and review articles dealing with the fundamental principles of stable isotope techniques, both from the instrumentation side and a physical chemistry point of view, which the interested reader is strongly encouraged to use for further study. These books and review articles are listed separately in the Recommended Reading section at the end of this book.

In the main, what follows will focus on the stable isotopes of the light elements of which all organic material is comprised, and why and how the stable isotope composition of an organic material can yield an added dimension of information with regards to "who, where and when".

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Part I
How it Works

Chapter I.1

What are Stable Isotopes?

Of the 92 natural chemical elements, almost all occur in more than one isotopic form, with the vast majority being stable isotopes, which do not decay, unlike radioisotopes, which are not stable and hence undergo radioactive decay. To put it another way, 61 of these 92 natural chemical elements appear in two or more stable isotopic forms. So, in this context "almost all" means with the exception of 20 stable chemical elements, including fluorine, sodium and phosphorus, which are mono-isotopic. Making up the difference between 81 and 92 are 11 naturally occurring radioactive chemical elements, including radon and technetium. The word isotope was coined by Professor Frederick Soddy at the University of Glasgow and borrows its origin from the two Greek words *isos* (ισοζ) meaning "equal in quantity or quality" and *topos* (τοποζ) meaning "place or position", with isotope hence meaning "in an equal position" (of the Periodic Table of the Elements). Incidentally, Frederick Soddy was awarded the Nobel Prize in Chemistry in 1921 for his work on the origin and nature of isotopes. By coining this term he referred to the fact that isotopes of a given chemical element occupy the same position in the Periodic Table of the Elements since they share the same number of protons and electrons, but have a different number of neutrons. The word isotope therefore does not denote radioactivity, as is so often mistakenly thought. As mentioned above, radioactive isotopes have their own name, radioisotope. Non-radioactive or stable isotopes of a given chemical element share the same chemical character and only differ in atomic mass (or mass number *A*), which is the sum of protons and neutrons in the nucleus.

Moving from the smallest entity upwards, atoms are composed of positively charged protons and neutral neutrons, which make up an atom's nucleus, and negatively charged electrons, which make up an atom's shell or electron cloud. Due to charge balance constraints, the number of protons is matched by the number of electrons. A chemical element and its position in the Periodic Table of the Elements is determined by the number of protons in its nucleus. The number of protons determines the number of electrons in the electron cloud and the configuration of this electron cloud in turn determines chemical characteristics such

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as electronegativity and the number of covalent chemical bonds a given element can form. Owing to this link, the number of protons in the atomic nucleus of a given chemical element is always the same and is denoted by the atomic number Z, while the number of neutrons (in its nucleus) may vary. Since the number of neutrons (N) has no effect on the number of electrons in the electron cloud surrounding an atom the overall chemical properties of an element are not affected. In other words, a chemical element like carbon will always behave like carbon irrespective of whether the number of neutrons in its nucleus is N or $N+1$. However, differences in mass-dependent properties can cause compounds containing different amounts of carbon with N or $N+1$ neutrons or at different positions to behave subtly differently, both chemically and physically.

Mass number A ($=Z+N$) and atomic number Z (= number of protons) are denoted as whole numbers in superscript and subscript, respectively, to the left of the element symbol. So carbon-12, comprising six protons and six neutrons is written as ${}^{12}_{6}\mathrm{C}$ while carbon-13, which comprises six protons and seven neutrons, is written as ${}^{13}_{6}\mathrm{C}$. In general practice different isotopes of the same chemical element are denoted by mass number and chemical symbol only, for example $^{2}\mathrm{H}$ or $^{13}\mathrm{C}$.

The simplest of chemical elements, hydrogen (H), in its most abundant isotopic form has a nucleus comprising a single proton and therefore has the atomic weight of 1 (in atomic mass units, amu) and this is indicated by adding a superscript prefix to the element letter, that is, $^{1}\mathrm{H}$. The less abundant and 1 neutron heavier hydrogen isotope is therefore denoted as $^{2}\mathrm{H}$, although one will also find the symbol D being used since this stable hydrogen isotope has been given the name deuterium. The discovery of this isotope won Harold C. Urey the Nobel Prize in Chemistry in 1934, and today Urey is regarded as one, if not *the* father of modern stable isotope chemistry.

Staying with hydrogen as example, one could say $^{1}\mathrm{H}$ and its sibling $^{2}\mathrm{H}$ are identical twins that have different weights and different abundances. In the case of hydrogen, the weight difference between the more abundant $^{1}\mathrm{H}$ and the less abundant $^{2}\mathrm{H}$ is one atomic mass unit. The same is true for the carbon twins. Here sibling carbon-13 ($^{13}\mathrm{C}$) is the heavier twin, weighing 1 amu more than its sibling carbon-12 ($^{12}\mathrm{C}$), and as is the case for the two hydrogen isotopes the heavier $^{13}\mathrm{C}$ is again the less abundant of the two stable carbon isotopes. However, in the case of carbon the actual weight difference of 1 amu amounts to a relative weight difference of only 8.33 % for $^{13}\mathrm{C}$ relative to $^{12}\mathrm{C}$ (cf. Table I.1) while in the case of hydrogen the weight difference of 1 amu means relative to $^{1}\mathrm{H}$ the less abundant isotope $^{2}\mathrm{H}$ is 100 % heavier than its twin, the more abundant isotope $^{1}\mathrm{H}$. Where the normal weight versus overweight twin analogy has its limitations is in the matter of abundance or occurrence, but only for as long as we stay with the example of two complete twins. We will revisit the twin example in the following chapter after a brief excursion through the natural abundance of stable isotope and natural abundance level variations.

Chapter I.2

Natural Abundance Variation of Stable Isotopes

The global, or perhaps better described as the mean, stable isotope abundances of all non-radioactive elements were set when the Earth was formed and, on a global or globally averaged scale have not changed significantly since. On the basis of globally averaged isotope abundances many a generation of students was taught that the stable isotope abundances of chemical elements were fixed constants and traditional analytical techniques did nothing to dispel this incorrectly held perception. While infrared spectroscopy, nuclear magnetic resonance spectroscopy and mass spectrometry are able to detect the presence of ^{13}C in a given organic compound, none of these techniques are sensitive enough to accurately detect let alone quantify subtle variations in stable isotope abundance at a level of 0.1 atom% or below. When I studied chemistry in the 1980s we were still taught that the natural abundance of ^{13}C was a constant 1.11 atom% even though the seminal work by Epstein and co-workers in the 1960s and 1970s had already demonstrated carbon isotopic fractionation and associated differences in ^{13}C composition of bio-organic compounds as a result of kinetic isotope effects during biochemical reactions (Deniro and Epstein, 1977; Park and Epstein 1960, 1961; Smith and Epstein, 1971).

The figures usually quoted in chemistry textbooks for isotope abundance refer to the globally averaged values, that is, when considering the entire carbon mass of the Earth system the natural abundances of ^{12}C and the one neutron heavier ^{13}C are 98.89 and 1.11 atom%, respectively (cf. Table I.1). However, what tends to be overlooked when referring to these abundance values and, hence, not be taught to students in chemistry classes is the fact that the compartmental isotope abundance of light elements is not fixed, but is in a continuous state of flux due to the mass discriminatory effects of biological, biochemical, chemical and physical processes. For instance, when looking at individual carbon pools one finds some with a higher abundance of ^{13}C, such as marine carbonate sediments, whereas others are more depleted in ^{13}C, such as the hydrocarbons found in crude oil.

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Table I.1 Key figures for stable isotopes of light elements.

Chemical element	Major abundant isotope[a]	First minor abundant isotope[a]	Second minor abundant isotope	Relative atomic mass difference First minor/major	Isotope ratio First minor/major for scale reference point
Hydrogen	^{1}H	^{2}H		100 %	0.00015576
	99.985 atom%	0.015 atom%			VSMOW
Carbon	^{12}C	^{13}C		8.33 %	0.0112372
	98.89 atom%	1.11 atom%			VPDB
Nitrogen	^{14}N	^{15}N		7.14 %	0.0036765
	99.63 atom%	0.37 atom%			Air
Oxygen	^{16}O	^{18}O	^{17}O	12.5 %	0.0020052
	99.76 atom%	0.20 atom%	0.04 atom%		VSMOW
Sulfur	^{32}S	^{34}S	^{33}S	6.25 %	0.0450045
	95.02 atom%	4.22 atom%	0.76 atom%		VCDT

[a]Note that listed isotope abundance values in atom% are global mean values on which atomic weights given in the old-style Periodic Table of the Elements are based (e.g. 1.00797 for H or 12.0112 for C); they are not identical and should thus not be confused with isotope abundance values or isotope ratios of the materials chosen as scale reference points.

The fact that atomic fractions or mole fractions of isotopes varied in normal naturally occurring materials was recognized in 1967 by the International Union of Pure and Applied Chemistry (IUPAC) Commission on Isotopic Abundances and Atomic Weights (CIAAW) or the Commission on Atomic Weights as it then was. Tables of Standard Atomic Weights published by CIAAW prior to 2009 listed only a single atomic weight for each element with at least one minor stable isotope. As of 2009, CIAAW started to report atomic weight intervals for some elements with the upper and lower boundaries of the atomic weight for a given chemical element, defining the interval within which the atomic weight value for this element in a given normal compound or material might be encountered. Standard atomic weight values for each chemical element are reviewed by CIAAW on a regular basis and the results of these reviews are reported to IUPAC and subsequently published as IUPAC Technical Reports in the journal *Pure and Applied Chemistry*. In the 2011 Table of Standard Atomic Weights the atomic weight interval for carbon was given as [12.0096, 12.0116] (Wieser *et al*., 2013). This change in reporting the atomic weights of chemical elements as atomic weight intervals is now also reflected in a new IUPAC Periodic Table of the Isotopes (Holden *et al*., 2011) a reproduction of which can be found in Figure I.1. It can be downloaded as a PDF from the CIAAW website (http://www.ciaaw.org/pubs/Periodic_Table_Isotopes.pdf).

Expressed in units of atom% and staying with the example of ^{13}C, these differences are very small, with the range covered amounting to approximately 0.11 atom%. To express these minute variations, the δ notation has been adopted to report changes in isotopic abundance compared to a designated isotopic standard

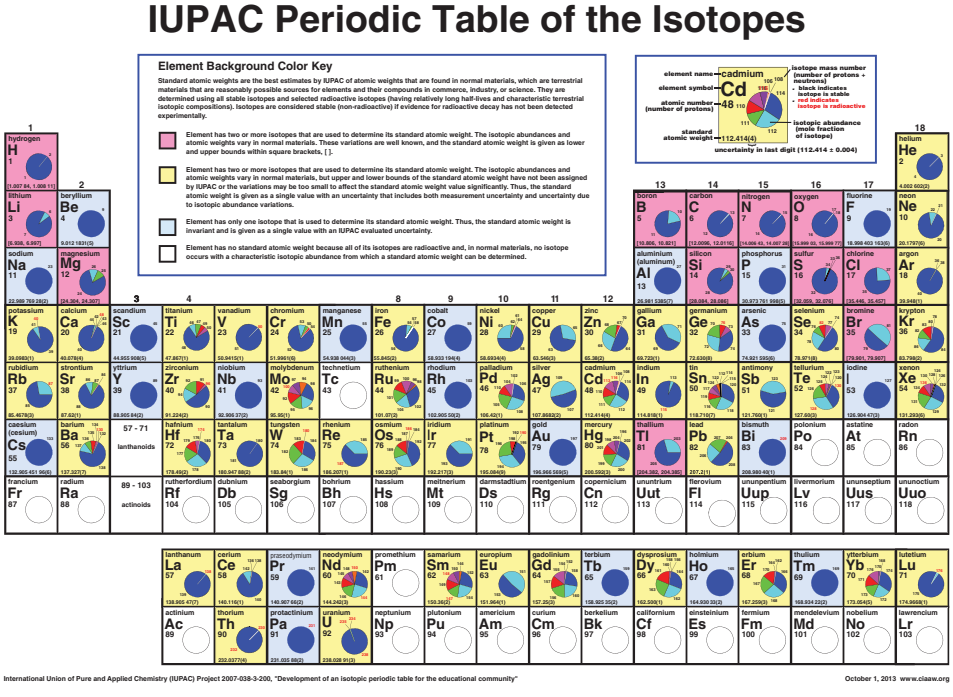

Figure I.1 New Period Table of Elements showing isotope abundance ranges. *Source:* Holden *et al.* (2011). Reproduced with permission of CIAAW.

(Equation I.1). In other words, δ values are relative isotopic abundance values suited for comparison, which is why it is crucially important to provide concise information on which δ value(s) and the nature of the scale anchor(s) whose δ values were employed to generate reported, scale normalized δ values (cf. Part II, Chapter II.3). In the most recent guidelines and recommended terms of stable isotope ratio measurements and reporting results thereof issued by IUPAC the δ value has been defined as follows (Coplen, 2011):

$$\delta^{\mathrm{h}}\mathrm{E}_{\mathrm{S}/\mathrm{STD}} = ([R_{\mathrm{S}} - R_{\mathrm{STD}}]/R_{\mathrm{STD}}) = ((R_{\mathrm{S}}/R_{\mathrm{STD}}) - 1) \tag{I.1}$$

In this equation, R_{S} is the measured isotope ratio of the heavier isotope h of a given chemical element E in a sample S over the lighter isotope l of the same element E (e.g. $^{13}\mathrm{C}/^{12}\mathrm{C}$ or $^{2}\mathrm{H}/^{1}\mathrm{H}$) and R_{STD} is the contemporaneously measured isotope ratio for the chosen standard (e.g. NBS 19 or Vienna Standard Mean Ocean Water, VSMOW). It should be noted the 2011 IUPAC definition of the δ value as stated in Equation I.1 no longer contains the extraneous factor of 1000. Since δ values derived on the basis of Equation I.1 are numerically less than zero with significant numbers to the second or third decimal place, they may be stated as ‰ values as a representation of the scientific notation of presenting such numbers as multiples of 10^{-3}. However, reporting δ values in this way should not lead the ‰ sign to be confused with an SI unit of measurement. As a ratio of two ratios δ values do not have an SI unit of measurement. Similar to the % symbol the ‰ symbol is not a unit (of measurement) but merely a convenient way to express small numbers.

For example, with $R_{\mathrm{S}} = 0.01101296$ and $R_{\mathrm{STD}} = 0.0112372$,

$$\delta^{\mathrm{h}}\mathrm{E}_{\mathrm{S}/\mathrm{STD}} = (0.980045 - 1) = -0.01995.$$

This δ value can be written as

$$\delta^{\mathrm{h}}\mathrm{E}_{\mathrm{S/STD}} = -0.01995 = -19.95 \times 10^{-3} = -19.95‰.$$

In this example the minus sign signifies that the isotopic abundance of $^{\mathrm{h}}\mathrm{E}$ in the sample S is less than the isotopic abundance of $^{\mathrm{h}}\mathrm{E}$ in the chosen reference material STD. Conversely, $\delta^{\mathrm{h}}\mathrm{E}_{\mathrm{S}/\mathrm{STD}}$ values with a positive sign signify an isotopic abundance of $^{\mathrm{h}}\mathrm{E}$ in the sample S that is higher than that in the reference material STD.

Using the δ notation, a difference in $^{13}\mathrm{C}$ abundance of 0.011 atom% corresponds to a change in $\delta^{13}\mathrm{C}$ value of 10 ‰. For example, a change in $^{13}\mathrm{C}$ abundance from 1.0893 atom% down to 1.0783 atom% corresponds to a change in $\delta^{13}\mathrm{C}$ value from −19.96 ‰ to −29.96 ‰ on the Vienna Pee Dee Belemnite (VPDB) scale. Owing to how accurately and precisely the $^{13}\mathrm{C}$ composition of homogeneous materials at the natural abundance level can be measured by modern analytical instruments, for organic materials measured differences > 0.3 ‰ are statistically significant. By comparison, the same difference of 0.011 atom% in the $^{2}\mathrm{H}$ abundance of a given compound corresponds to a change in $\delta^{2}\mathrm{H}$ value of approximately 706 ‰.

For example, a change in ^{2}H abundance from 0.016 atom% down to 0.005 atom% corresponds to a change in $\delta^{2}H$ value from +27.4 ‰ to −679.0 ‰ on the VSMOW scale. Yet a difference in ^{2}H abundance of 0.001 atom% corresponds to a change in $\delta^{2}H$ value of approximately 64 ‰ and not 70.6 ‰ as one might have expected.

The above examples illustrate that the δ notation or δ value is not a linear function of isotopic abundance since isotopic abundance is an atomic fraction (or a mole fraction). However, at near natural abundance levels where, for example, the ^{13}C atomic fraction ranges from 0.010 to 0.012 the deviation of $\delta^{13}C$ values from linearity when plotted against ^{13}C atomic fractions is virtually non-existent. Conversely, when dealing with samples enriched in ^{13}C and their ^{13}C atomic fraction exceeds 0.1, this non-linearity cannot be ignored. Using $\delta^{13}C$ values for, for example, isotope mass balance calculations as an approximation of isotopic abundance for atomic fractions in excess of 0.1 would introduce significant errors (Fry, 2006).

The δ notation or δ value as defined in Equation I.1 is a ratio and thus a derived quantity without a unit of measurement. Following tradition, in the majority of publications δ values are expressed in "units" of parts per thousand and communicated as per mil values (symbol "‰"). The "‰" symbol is set here in quotation marks to stress the point already made above that symbols such as the per cent sign (%) or the per mil sign (‰) are not units of measurement as defined in the International System of Units (SI) like, for example, meter (m), joule (J) or Pascal (Pa). To bring the δ notation or δ value in line with the International System of Units, in 2012 CIAAW members Willi Brand and Tyler Coplen proposed the introduction of a new unit for this derived quantity defined through Equation I.1 (Brand and Coplen, 2012). They proposed this new SI unit could have the name Urey (symbol Ur), in recognition of Harold C. Urey, who in 1934 received the Nobel Prize in Chemistry for his discovery of the hydrogen isotope ^{2}H. Their argument was that this new unit would follow the example set by the SI unit for pressure, the Pascal, unit symbol Pa, named after Blaise Pascal. The new Urey unit could be combined with any SI prefix used to express fractions or multiples of 10 of an SI unit. A δ value of 1 ‰ would thus become a δ value of 1 milliurey (symbol mUr).

Various isotope scale reference points are used for reporting the relative isotopic abundance of the light elements hydrogen, carbon, nitrogen, oxygen and sulfur. By virtue of Equation I.1, the δ values of each of these scale reference points are by definition 0. Carbon stable isotope ratios were originally reported relative to the reference material Pee Dee Belemnite (PDB), which also served as scale reference point. Since this reference material became exhausted, VPDB has become the new international scale reference point for the ^{13}C scale. Oxygen stable isotope abundance values of carbonates and calcites are also commonly expressed relative to VPDB. However, unlike the old scale anchor PDB the new scale anchor VPDB is a virtual reference material that does not exist in a material form. Instead NBS 19, a limestone material composed largely of calcite, is used to underpin the VPDB scale (cf. Table I.2). Sulfur stable isotope abundance values are scale anchored in a similar way. Originally the ^{34}S scale was anchored by Troilite (FeS) from the

Table I.2 Scale reference points and their defining scale anchors for stable isotopes of light elements.

Stable isotope	Scale reference point	Scale anchors	Chemical compound	Relative stable isotope abundance value as ‰
Hydrogen	VSMOW	VSMOW2	Water	0.0
		SLAP2	Water	−427.5
Carbon	VPDB	NBS 19	Calcite	+1.95
		LSVEC	Li_2CO_3	−46.60
Nitrogen	Air	IAEA-N-1[a]	$(NH_4)_2SO_4$	+0.43
		USGS32[a,b]	KNO_3	+180.0
Oxygen	VSMOW	VSMOW2	Water	0.00
		SLAP2	Water	−55.50
Sulfur	VCDT	IAEA-S-1	Ag_2S	−0.3
		IAEA-S-2	Ag_2S	+22.62

[a]Cf. page 439 of the IUPAC technical report by W.A. Brand *et al*. (2014) *Pure and Applied Chemistry*, **86**(3), 425–467.

[b]Recommendation that is still subject to confirmation by IUPAC.

Cañon Diablo meteorite (CDT) until questions arose about variability of its $\delta^{34}S$ values and the cause for this variability. In 1997 the decision was therefore taken to henceforth express $\delta^{34}S$ values on the Vienna Cañon Diablo Troilite (VCDT) scale, with IAEA-S-1 (silver sulfide) being used to underpin the VCDT scale (Krouse and Coplen, 1997). Like VPDB, VCDT does not exist but is a virtual isotopic reference material anchored by IAEA-S-1 (cf. Table I.2).

Stable oxygen and hydrogen isotopic abundance values are reported relative to VSMOW (Vienna Standard Mean Ocean Water) which is anchored by two scale anchors, VSMOW and SLAP (Standard Light Antarctic Precipitation). Nitrogen isotope abundance values are reported relative to Air (for nitrogen in atmospheric air).

The use of VSMOW and VPDB as scale reference points when reporting δ values means that measurements have been normalized according to International Atomic Energy Agency (IAEA) and IUPAC guidelines for expression of δ values relative to traceable reference materials on internationally agreed reference scales (Coplen *et al*., 2006a,b; Coplen, 2011). Table I.3 lists a number of international reference materials recommended for use as scale anchors and/or quality control materials. Scale normalization of measured δ values will be discussed in detail in Part II, Chapter II.3. The international reference materials listed in Table I.3 are available from the IAEA,[1] the National Institute of Standards and Technology (NIST),[2] and the United States Geological Survey (USGS).[3]

[1] https://nucleus.iaea.org/rpst/referenceproducts/referencematerials/Stable_Isotopes/index.htm.

[2] https://www-s.nist.gov/srmors/viewTableV.cfm?tableid=42.

[3] http://isotopes.usgs.gov/lab/referencematerials.html.

Table I.3 A representative but not exhaustive list of international reference materials for stable isotope ratio mass spectrometry together with their stable isotope abundance values as published by the Commission on Isotopic Abundances and Atomic Weights (CIAAW; http://www.ciaaw.org/reference-materials.htm).

International Reference Material (IRM)	Code	$\delta^{13}C_{VPDB}$ as ‰	$\delta^{15}N_{AIR}$ as ‰	$\delta^{2}H_{VSMOW}$ as ‰	$\delta^{18}O_{VSMOW}$ as ‰
TS-limestone	NBS 19	+1.95			+28.65[a]
Carbonatite	NBS 18	−5.01			+7.20[a]
Lithium carbonate	LSVEC	−46.6			+3.69[a]
Oil	NBS 22	−30.03		−116.9	
Sucrose	IAEA-CH-6	−10.45			
Polyethylene foil	IAEA-CH-7	−32.15		−100.3	
Caffeine	IAEA-600	−27.77	+0.91		−3.48
L-glutamic acid	USGS40	−26.39	−4.52		
L-glutamic acid	USGS41a	+36.55	+47.55		
Cellulose	IAEA-CH-3	−24.72			
Ammonium sulfate	IAEA-N-1		+0.43		
Ammonium sulfate	IAEA-N-2		+20.35		
Potassium nitrate	USGS32		+180.0		+25.4
Potassium nitrate	USGS34		−1.8		−27.78
Water	VSMOW			0	0
Water	VSMOW2			0	0
Water	GISP			−189.7	−24.78
Water	GISP2			−258.3	−33.43
Water	SLAP			−428.0	−55.5
Water	SLAP2			−427.5	−55.5
Water	IAEA-604			+799.9	−5.86
Benzoic acid	IAEA-601	−28.81			+23.14
Benzoic acid	IAEA-602	−28.85			+71.28

[a]Traditionally, $\delta^{18}O$ values for carbonates are reported on the VPDB scale. The $\delta^{18}O_{VPDB}$ values for NBS 19, NBS 18 and LSVEC are +2.20, −23.01 and −26.41 ‰, respectively.

Let us now revisit the twin analogy to picture what natural abundance means in praxis. Obviously, the abundance ratio of any given pair of twins is 1:1 or 50 %:50 %, that is, when meeting any one twin in a crowd where both are known to be present, one has an even chance of speaking either to twin A or twin B. However, if we consider a hypothetical case where both twins were victims of a major explosion, the probability of any given body part belonging to either twin now becomes a function of the number of pieces each body has been divided into. The same in a way is true for chemical elements and their "overweight" twins. If one took apart a lump of sugar to its molecular level, one would find that, depending on circumstances (in this case which plant had produced the sugar), one would have a 98.9617 % or a

98.9015 % chance of finding ^{12}C if the sugar was beet sugar or cane sugar, respectively. Similarly, one would have a 1.0833 % or 1.0985 % chance of finding ^{13}C in carbon from beet sugar and cane sugar, respectively. So, generally speaking, one always has a better chance of encountering ^{12}C than ^{13}C, meaning ^{12}C has a higher abundance than its heavier isotope ^{13}C. However, on a case-by-case basis one finds that chemically identical substances such as sugar may exhibit different isotopic compositions where a change in ^{12}C abundance is accompanied by a proportionate yet opposite change in ^{13}C. For example, beet sugar contains more ^{12}C and less ^{13}C than cane sugar while conversely cane sugar contains more ^{13}C and less ^{12}C than beet sugar (Hobbie and Werner, 2004; Meier-Augenstein, 1999; Rossmann *et al*., 1997). The chemical and physicochemical reasons behind these differences will be discussed in Chapters I.3 and I.4.

Chapter I.3

Chemically Identical and Yet Not the Same

The analytical methods traditionally applied in forensic science laboratories establish a degree of commonality between one substance and another by identifying their constituent elements and functional groups, and elucidating their chemical structures. Thus, for two samples of sugar all of the aforementioned data will correspond and it can be concluded that they are chemically indistinguishable, they are indeed both sugar. However, it can be argued that although the two substances in question are chemically indistinguishable they may not be the same, for example they may have come from different sources or be of different origin. Attention is drawn to the following: whenever we speak of the source and origin of a natural product such as sugar, by source we mean from which particular plant was the sugar sourced (i.e. ultimately made) whereas by origin we mean its geographic origin (i.e. where the plant was grown and harvested). In other words by differentiating between source and/or origin the distinction is being made that if two substances do not share the same provenance they are not truly identical even if chemically they are indistinguishable. This assertion can be contested by stable isotope analysis either to protect people from being convicted of a crime they have not committed, such as drug trafficking, or, staying with the example of drugs, to convict people who may be prepared to admit to the lesser offence of possession for personal use while in fact they are drug dealers or drug traffickers.

How is this possible? For reasons we will touch upon in this chapter and again in Chapter I.4, two chemically indistinguishable compounds will be isotopically distinguishable if they do not share the same compound history, that is, if the processes involved in their synthesis differ, which for biogenic natural compounds could mean they were derived from a different source (plant or organism) or even from an identical source but with a different geographic origin. Using the case of sugar as example, traditionally the two main sources of household sugar are sugar cane and sugar beet. With the help of stable isotope signatures it is perfectly straightforward to determine

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Table I.4 Select examples of ^{13}C and ^{2}H abundance values of sugar from different plant sources and of different geographic origin.

Sample	$\delta^{13}C_{VPDB}$ as ‰	$\delta^{2}H_{VSMOW}$ as ‰
Sugar (sugar beet, Poland)	−25.42	−71.0
Sugar (sugar beet, Sweden)	−26.84	−93.4
Sugar (sugar cane, Brazil)	−11.76	−21.4
Sugar (sugar cane, South Africa)	−11.10	−6.7

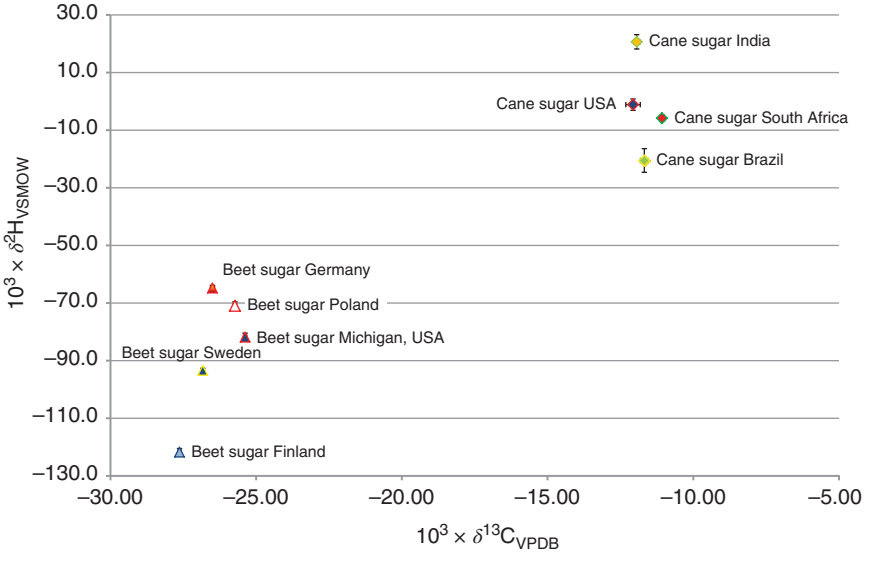

Figure I.2 $\delta^{2}H$ and $\delta^{13}C$ values of beet sugar and cane sugar of different geographic origin.

if a given sugar sample is either cane sugar or beet sugar. In addition, it is even possible to say where approximately in the world the sugar cane or sugar beet was grown and cultivated (cf. Table I.4 and Figure I.2).

The differences in isotopic composition between cane sugar and beet sugar are ultimately caused by differences in biochemical reactions where chemical bonds are broken and formed, and differences in the isotopic compositions of precursor pools. The latter can be the result of physicochemical processes. Even though these processes do not involve the formation or break-up of chemical bonds they nonetheless reflect differences in bond length and bond strength that can result in significant differences in compound properties such as melting point or boiling point. The isotopologues of water are an excellent example of this phenomenon

Table I.5 Influence of isotopic composition on physical properties of H_2O and its isotopologues.

Property	$^1H_2{}^{16}O$ FW = 18 g/mole	$^1H_2{}^{18}O$ FW = 20 g/mole	$^2H_2{}^{16}O$ FW = 20 g/mole
Reduced mass μ of H–O bond (amu)	0.9412	0.9474	1.7778
Melting point (°C) at 101.33 kPa	0	0.28	3.82
Boiling point (°C) at 101.33 kPa	100	100.15	101.4
Vapour pressure at 20 °C in kPa	2.3379	2.3161	2.0265
Heat of vaporization (kJ/mole) at 100 °C	40.657	(40.664)[a]	41.521
Neutral pH at 25 °C	7	(7.004)[a]	7.47
Dissociation constant pK_w ($mole^2/kg^2$) at 25 °C	14	(14.008)[a]	14.957
O–H bond length (Å)	0.990	(0.9899)[a]	0.9846

[a]No official values are available. Values in brackets are interpolated approximations.

(cf. Table I.5). In Chapter I.4 we will learn about why isotopologues, that is, molecules of the same compound but of different isotopic composition (Sharp, 2007), behave and react in subtly different ways even though on an atomic level the heavy and light isotopes of the same chemical element behave in the same way.

Chapter I.4

Isotope Effects, Mass Discrimination and Isotopic Fractionation

I.4.1 Physical Chemistry Background

If for a given compound a non-quantitative chemical reaction or a physicochemical process such as vaporization has taken place, this will be subject to mass discrimination (or associated with an isotope effect), which will cause a change in isotope abundance and hence result in isotopic fractionation. In principle two different types of isotope effects can cause isotopic fractionation: kinetic isotope effects (kinetic as in chemical reaction kinetics) and thermodynamic isotope effects. In general, mass discrimination is caused by differences in the vibration energy levels of bonds involving heavier isotopes as compared to bonds involving lighter isotopes.

Differences in the zero-point energy of chemical bonds containing one heavy isotope and one light isotope relative to bonds containing two light isotopes are reflected by differences in the rates of cleavage of these bonds because differences in zero-point energy results in differences in bond energy. For example, for hydrogen gas the bond strengths of ^{1}H–^{1}H, ^{1}H–^{2}H and ^{2}H–^{2}H are 436.0, 439.4 and 443.5 kJ/mole, respectively. Thus, ^{2}H–^{2}H bonds are broken at a slower rate than ^{1}H–^{2}H bonds, which in turn are broken at a slower rate than ^{1}H–^{1}H bonds. It is usually observed that the product of a chemical reaction involving bond cleavage will be isotopically lighter in the element(s) forming that bond compared to the corresponding isotopic composition of the initial precursor or source substrate.

It might be useful at this point to revisit some basic principles of physics and a mainstay of analytical chemistry instrumentation, infrared (IR) spectroscopy, to illustrate the relation between the reduced mass of a chemical two-atom system, bond length and bond strength aka bond energy, which are ultimately responsible

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for the mass discrimination that leads to the wide range of isotopic composition of natural and man-made compounds.

The rotational (or vibrational) kinetic energy E_{vib} of a rigid body can be expressed in terms of its moment of inertia I and its angular velocity ω:

$$E_{vib} = \frac{1}{2} I\omega^2 \tag{I.2}$$

The (scalar) moment of inertia I of a point mass m rotating about a known axis r is defined by

$$I = mr^2 \tag{I.3}$$

For a system comprising two masses (or two atoms) m_1 and m_2 joined by, say, a spring (or chemical bond) of the length r, and if this system rotates around an axis intersecting a point on that spring (or bond), the mass term m in Equation I.3 is replaced by the reduced mass μ of this system, which is given by

$$\mu = \frac{m_1 m_2}{m_1 + m_2} \tag{I.4}$$

The vibrational or rotational energy of a molecule can be measured by its IR absorbance. In the world of quantum physics where rotating or vibrating systems assume discreet energy levels, the associated discreet packets of energy differences ΔE can be expressed by the rotational constant B, the difference between two IR absorption bands:

$$\Delta E_{\Delta J=1} = 2B = \frac{h}{4\pi^2 cI} \tag{I.5}$$

Here h is the Planck constant and c is the speed of light.

As mentioned in Chapter I.3, the different isotopologues of water are a good example of how differences in bond strength affect the physicochemical properties of a compound (cf. Table I.5). IR spectrometry-based isotope analysers manufactured and marketed by companies such as Los Gatos, Picarro or Thermo Fisher Scientific exploit differences in IR active vibrational energy states between isotopologues of CO_2 to conveniently measure the abundance of, for example, atmospheric $^{13}CO_2$ as a tool for greenhouse gas monitoring. These laser-based systems operate either in the near-IR or the mid-IR part of the IR spectrum. In the near-IR, distance in units of wavenumbers (cm^{-1}) between absorption lines of the different isotopologues are quite small, for example <1 cm^{-1}, with absorption lines for $^{12}CO_2$ and $^{13}CO_2$ being found at 6251.75 cm^{-1} and 6251.30 cm^{-1} respectively. Moving into the mid-IR these differences become more pronounced, for example >20 cm^{-1}, with absorption lines for $^{12}CO_2$ and $^{13}CO_2$ found at 2325.58 cm^{-1} and 2247.19 cm^{-1}, respectively.

The IR spectra of gaseous hydrochloric acid (HCl) are another fine example of how differences in isotopic make-up and therefore differences in μ and r, and hence in I, result in differences in ΔE between neighbouring IR absorption bands for, for example, ^{1}H–^{35}Cl and ^{2}H–^{35}Cl. Since we are able to measure B and can calculate μ it is possible to calculate r, the bond length for the different HCl isotopologues.

Question I.1 Given that values for B for ^{1}H–^{35}Cl and ^{2}H–^{35}Cl have been measured to be 10.44 cm^{-1} and 5.39 cm^{-1}, respectively, what bond lengths would one calculate for a ^{1}H–^{35}Cl molecule and a ^{2}H–^{35}Cl molecule? Note: To calculate the reduced mass μ of one molecule one has to divide the reduced mass term (g/mole) by Avogadro's number (molecules/mol). For the Planck constant h use 6.62×10^{-34} Js and for the speed of light c use 3×10^{8} m s^{-1}.

The values for B for ^{1}H–^{37}Cl and ^{2}H–^{37}Cl are 10.42 cm^{-1} and 5.38 cm^{-1}, respectively.

I.4.2 Fractionation Factor α and Enrichment Factor ε

The difference in bond length and hence bond strength between bonds involving different isotopes of the same chemical element that already results in measurable differences in spectroscopic characteristics also leads to different reaction rates for a bond when different isotopes of the same element are involved (Melander and Saunders, 1980). The most significant isotope effect is the kinetic or primary isotope effect, whereby a bond containing the chemical elements under consideration is broken or formed in the rate-determining step of the reaction (Rieley, 1994), for example the reaction between two amino acids leading to the formation of the peptide bond R–CO–NH–R′ involving the carboxyl carbon of amino acid R and the amino nitrogen of amino acid R′.

The second type of isotope effect is associated with differences in physicochemical properties such as IR absorption, molar volume, vapour pressure, boiling point and melting point. Of course, these properties are all linked to the same parameters as those mentioned for the kinetic isotope effect, that is, bond strength, reduced mass and hence vibration energy levels. However, to set it apart from the kinetic isotope effect, this effect is referred to as *thermodynamic isotope effect* (Meier-Augenstein, 1999) because it manifests itself in processes where chemical bonds are neither broken nor formed. Typical examples for such processes in which the results of thermodynamic isotope effects can be observed are IR spectroscopy and any kind of two-phase partitioning (e.g. liquid/liquid extraction) or phase transition (e.g. liquid to gas, i.e. distillation or vaporisation). The thermodynamic isotope effect, or physicochemical isotope effect, is the reason for the higher IR absorption of $^{13}CO_2$ as compared to $^{12}CO_2$, for the vaporization of ocean surface water resulting in clouds (= water vapour) being depleted in both ^{2}H and ^{18}O compared to ocean surface water, and for the isotopic fractionation observed during chromatographic separations.

Another way of describing any isotope effect is to say that the reaction rate constant or equilibrium constant k of a given reaction or transformation

$$\text{Precursor (or Source)} \xrightarrow{k} \text{Product}$$