

Stable Isotope Forensics

An Introduction to the Forensic Application
of Stable Isotope Analysis

Wolfram Meier-Augenstein

Centre for Anatomy & Human Identification

University of Dundee, UK

SCRI, Invergowrie, Dundee, UK



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Series Foreword

Developments in Forensic Science

The world of forensic science is changing at a very fast pace. This is 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 be a valuable resource for forensic science practitioners in the pursuit of such knowledge.

The Forensic Science Society is the professional body for forensic practitioners in the United Kingdom. The Society was founded in 1959 and gained professional body status in 2006. The Society is committed to the development of the forensic sciences in all of its many facets, and in particular to the delivery of highly professional and worthwhile publications within these disciplines through ventures such as this book series.

Dr Niamh Nic Daéid

Series Editor

Foreword

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

The breadth of material covered 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 which 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

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Introduction

Stable Isotope 'Fingerprinting' 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 is a deliberate analogy to the term 'DNA Fingerprinting' coined by Professor Sir Alec J. Jeffreys. I seek 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, and human provenancing in particular. At the same time, it has also been 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. One could argue that the random match probability of 1 : 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 were also seemingly matched with multivariate or multifactor probabilistic equations 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 isotope given in δ units of per mil (‰) (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 (^{13}C) is 110‰ (Fry, 2006), so for the purpose of this example we could say keratin can assume 110 different integer ^{13}C 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 : 638 million to 1 : 103.95 billion. In fact, one can calculate that the analysis of hair keratin for its isotopic composition with regard to hydrogen, carbon, nitrogen, oxygen and

sulfur would theoretically yield a combined specificity of 1 : 1 billion, thus suggesting a 'stable isotope fingerprint' based on these four letters of the chemical alphabet may have 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 should 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 actually assumed natural abundance ranges of organic materials are usually much narrower than the widest possible range. We will learn more about that in the course of this book. Thus, forensic scientists and statisticians such as Jurian Hoogewerff and Jim Curran suggest more conservative estimates, and put the potentially realized random match probability of stable isotope fingerprints at levels between 1 : 10 000 and 1 : 1 million, depending on the nature and history of the material under investigation. However, even at these lower levels, stable isotope profiling is a potentially powerful 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	^2H
C	^{13}C
G	^{15}N
T	^{18}O
[U]	[^{34}S]

Random match probability of Biological DNA is approximately 1 : 1 billion (1×10^9) for a DNA profile based on 10 loci.

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 : 1.04×10^{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 isotopic abundances of ^2H , ^{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 ^2H and ^{18}O , which when originating both from water 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 units, its range in a particular material such as coca leaves may only extend to 7 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 – they do not change over time. Drawing on an example from environmental forensics, calling a gas chromatography or gas chromatography-mass spectrometry 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 compound will have changed as 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 can the stable isotopic composition of the residual sample 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 the stable isotope signature does or does not reveal.

Despite these caveats it is easy to see why the prospect of potentially 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; however, 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 research and, in turn, becomes 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 this book we still have a mountain to climb if we are 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, storage and preparation through to the analytical measurement and 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 upcoming generations of forensic scientists in this technique as early as possible. Fortunately, in spite of the aforementioned drawbacks, this is possible for two main reasons; (i) Thanks to end-user interest, there is a sufficient amount 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 ranging from archaeology, biochemistry, environmental chemistry, geochemistry, palaeoecology to 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 of this analytical technique. To this end some of the physical chemistry background relating to such aspects as 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 back of this book.

In the main, what follows will focus on stable isotopes of light elements of which all organic material is comprised, and why and how stable isotope composition of an organic material can yield an added dimension of information with regard to 'Who, Where and When?'

References

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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 – the vast majority of these being stable isotopes, which do not decay, unlike radioisotopes, which are not stable and, hence, undergo radioactive decay. In this context, ‘almost all’ means with the exception of 21 elements, including fluorine and phosphorous, which are mono-isotopic. The word isotope was coined by Professor Frederick Soddy at the University of Glasgow, and borrows its origin from the two Greek words *isos* (ἴσος) meaning ‘equal in quantity or quality’ and *topos* (τοπος) meaning ‘place or position’, with isotope thus meaning ‘in an equal position’ (of the periodic table of chemical elements). Frederick Soddy was later 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 elements since they share the same number of protons and electrons, but have a different number of neutrons. Therefore, as is so often mistakenly thought, the word isotope does not denote radioactivity. As mentioned above, radioactive isotopes have their own name – radioisotopes. 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 comprised 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 (‘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 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 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