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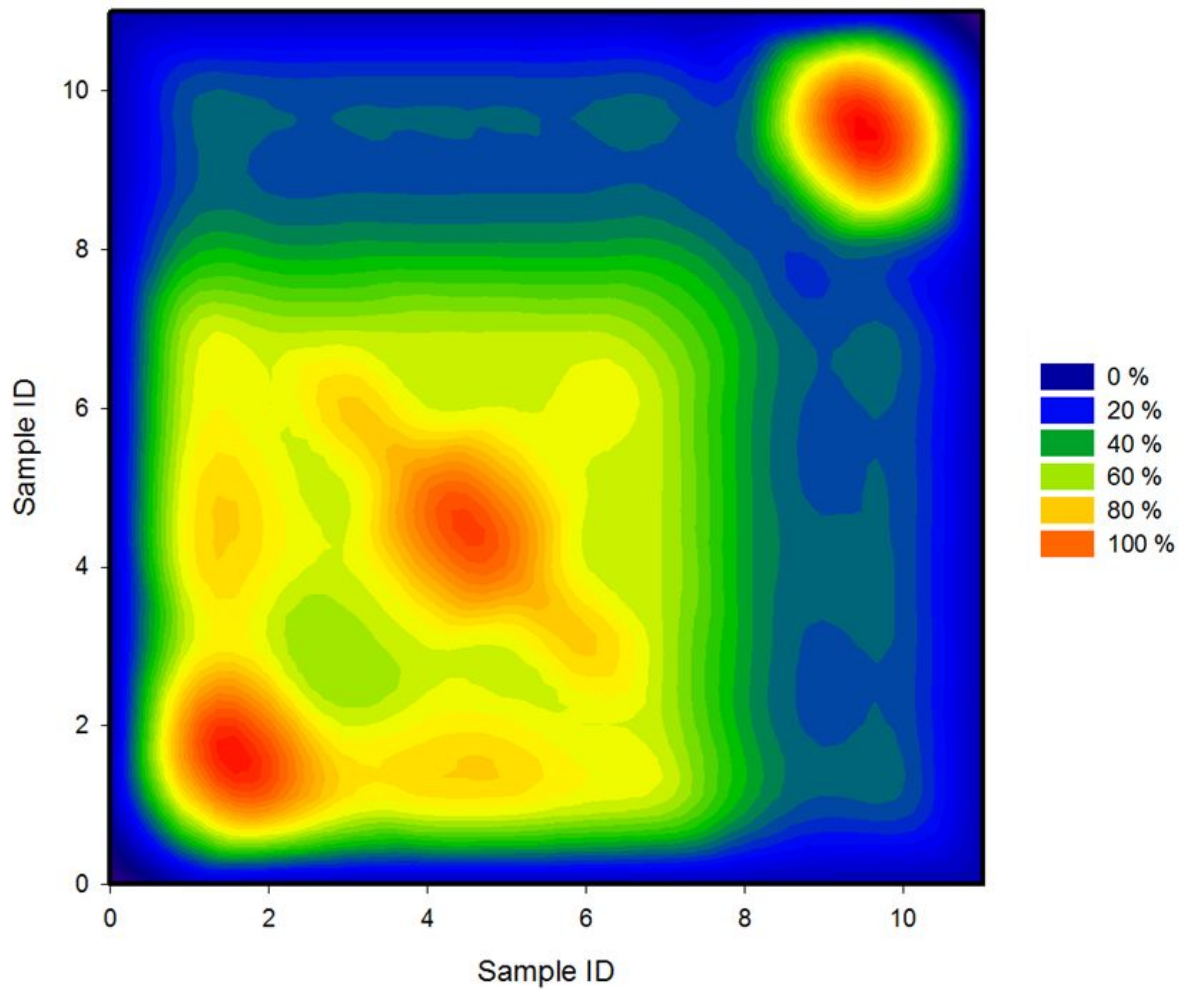
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**Forensic stable isotope signatures: comparing, geo-locating,
detecting linkage**

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38 Example of an *m versus n* plot showing topography of similarity levels between seized samples (#1
39 to #8) and controls (#9 and #10) as a visual means to illustrate strength of evidence to a jury.
40 Sample *m* is compared to sample *n* for all possible combinations meeting the condition $m \neq n$.
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Forensic stable isotope signatures: comparing, geo-locating, detecting linkage.

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Abstract

Stable isotope signatures or profiles of physical evidence such as illicit drugs, explosives or human tissue provide information on source, origin, even sample history not obtainable by traditionally applied analytical techniques of forensic chemistry. The discriminatory power, calculated as random match probability, of multivariate stable isotope signatures able to distinguish two cocaine samples from different regions in Colombia can range from one in tens of thousands to one in several million if based on the stable isotope abundances of carbon, nitrogen and hydrogen or carbon, nitrogen, hydrogen and oxygen respectively. Stable isotope signatures of physical evidence have therefore at the very least great potential to provide invaluable forensic intelligence for intelligence led policing. They may even be of great evidentiary value, especially if corroborated by results from independent analytical techniques. This review aims to offer a glimpse into the fascinating world of forensic stable isotope analysis by discussing the various levels of information stable isotope signatures can provide. For reasons easily appreciated, only a select few instances of its application to criminal investigations have been reported in scientific journals thus far. The various applications of this technique presented in this review are therefore predominantly taken from peer-reviewed work published in scientific books and journals.

1 Introduction

Earliest published work applying stable isotope analysis to compounds of interest in a forensic context dates back to 1975 when a study was published reporting differences in ^{13}C isotopic composition at natural abundance level of the high explosive trinitrotoluol (TNT), (Nissenbaum, 1975). Yet, in terms of forensically driven work involving stable isotope analytical techniques being published in a continuous fashion, the dawn of stable isotope forensics dates back to the 1990ies. Of the six articles published five dealt with stable isotope analysis of controlled substances, i.e. narcotic drugs (Besacier, Chaudron-Thozet, Lascaux, & Rousseau-Tsangaris, 1999; Besacier, Chaudron-Thozet, Rousseau-Tsangaris, Girard, & Lamotte, 1997; Besacier, Guilluy, Brazier, Chaudron-Thozet, Girard, & Lamotte, 1997; Desage, Guilluy, Brazier, Chaudron, Girard, Cherpin et al., 1991; Ehleringer, Cooper, Lott, & Cook, 1999) and one aimed to demonstrate proof-of-principle by reporting stable isotope abundance data from a variety of compounds of forensic interest (Avak, Hilkert, & Pesch, 1996). Since then interest has increased steadily in the benefits stable isotope abundance data or stable isotope signatures can bring to forensic science in general and criminal investigations in particular. The growing interest in and increasing appreciation of this technique is reflected not only by the overall number of peer reviewed publications having risen to almost 1,000 by the end of 2018 but also by the number of law enforcement agencies or associated forensic science service agencies that have set up forensic stable isotope laboratories. Amongst the organisations that have done so are well known names such as the Bureau of Alcohol, Tobacco, Firearms and Explosives (BATF), the Counter Terrorism Forensic Science Research Unit of the Federal Bureau of Investigation (FBI), the Drug Enforcement Administration (DEA) in the USA and the Australian Forensic Drug Laboratory at the National Measurement Institute, Australia, to name but four.

This review aims to portray the different facets of the forensic stable isotope analytical spectrum as they have evolved during the last 20 years in response to investigative needs as well as demands on scientific quality and robustness of the data or answers generated. In a parallel strand, making use of the examples discussed, this review also attempts to offer an answer to the question if stable isotope signatures should be used to serve as forensic intelligence tool in support of intelligence led policing or should their use focus on being given in evidence in a court of law.

2 Comparative analysis

One form of comparative testing may involve stable isotope analysis of a given sample and comparison of results against a data base. This approach is particularly well suited when a stable isotopic signature can be used as exclusion criterion to enable decision making.

2.1 Presumptive testing

Comparative analysis can be used and has been used as a presumptive test of authenticity of plant derived food and flavours. Used in the context of authenticity testing, stable isotope signatures are best as exclusion criteria as illustrated by the following example. A quick bulk ^{13}C analysis of a drop of vegetable oil may be all that is needed to reject a vegetable oil sold as 100 % maize germ oil to flag it as adulterated or at least to remove it from sale as being of questionable authenticity until further analyses have been carried out. Maize is a C_4 plant which means pure maize oil should show a bulk $\delta^{13}\text{C}$ value in the range of -16 to -10 ‰. By contrast, vegetable oils derived from C_3 plants

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3 exhibit $\delta^{13}\text{C}$ values in the range of -32 to -26 ‰. Therefore any vegetable oil sold as 100 % maize oil
4 showing a $\delta^{13}\text{C}$ value of less than -14 ‰ would have to be declared suspicious because such a finding
5 would mean this oil comprises at best a mixture of maize oil and other seed oils as a study published
6 in 1994 has shown (Rossell, 1994). The UK's Ministry of Agriculture, Fisheries and Food (MAFF as
7 then was) applied the insights gained from Rossell's study in a survey carried out in 1995 of
8 vegetable oils labelled and sold as 100 % maize oil (Ministry of Agriculture Fisheries and Food, 1995).
9 MAFF's survey found that based on their $\delta^{13}\text{C}$ values 35 % of all vegetable oils analysed contained
10 undeclared vegetable oils from other plant sources at a level of at least 3 %. Undeclared vegetable
11 oils in these mixed oils were typically soya oil or rapeseed oil.
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15 In food authenticity control, e.g. when testing for signs of fraudulent food adulteration,
16 comparative stable isotope abundance analysis is best suited as decision enabling method based on
17 tested foods meeting certain acceptance criteria or exclusion criteria for stable isotopic composition
18 of one or more key compounds. To name but one example, both the European Association of the
19 Industry of Juices and Nectars (AIJN) and the Comité Européen de Normalisation (CEN) have adopted
20 this approach based on a method determining the stable isotope signature of water in fruit juice to
21 distinguish between 100 % squeezed or pressed fruit juice, i.e. 100 % fruit juice not made from
22 concentrate and 100 % fruit juice made from concentrate. This method of determining the stable
23 isotope signature of water as an acceptance or exclusion criterion of food authenticity has also been
24 adopted by the International Organisation of Vine and Wine (OIV) (The International Organisation of
25 Vine and Wine, 2009).
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30 **2.2 Narcotic drugs and excipients**

31 In contrast to its application in forensic food analysis, comparative stable isotope analysis of narcotic
32 drugs or excipients (bulking or cutting agents) may serve two purposes; (i) Disproving a link or
33 connection between two samples of seized drugs (see section 2.2.2); (ii) Indicating a link or
34 connection between two samples of seized drugs. The results of comparative ^{13}C and ^{15}N abundance
35 analysis of seized carfentanil samples is a relatively recent example of the usefulness of this
36 approach in the fight against illicit drugs, especially a drug like fentanyl that is linked to a rising
37 number of overdose deaths (Casale, Mallette, & Guest, 2017). Either result will provide powerful
38 forensic intelligence helping to focus investigative efforts and allocate police resources accordingly.
39 In addition, as has happened on a few occasions, suspects or defendants have changed their plea of
40 not-guilty to a plea of guilty when presented with the results of multivariate stable isotope analyses.
41 However, in the majority of investigations the author has been involved with stable isotope
42 signatures of drugs such as amphetamine, Ecstasy and heroin or drug cutting agents such as
43 benzocaine, the primary objective was to generate forensic intelligence for law enforcement
44 agencies. Three examples are briefly reviewed below.
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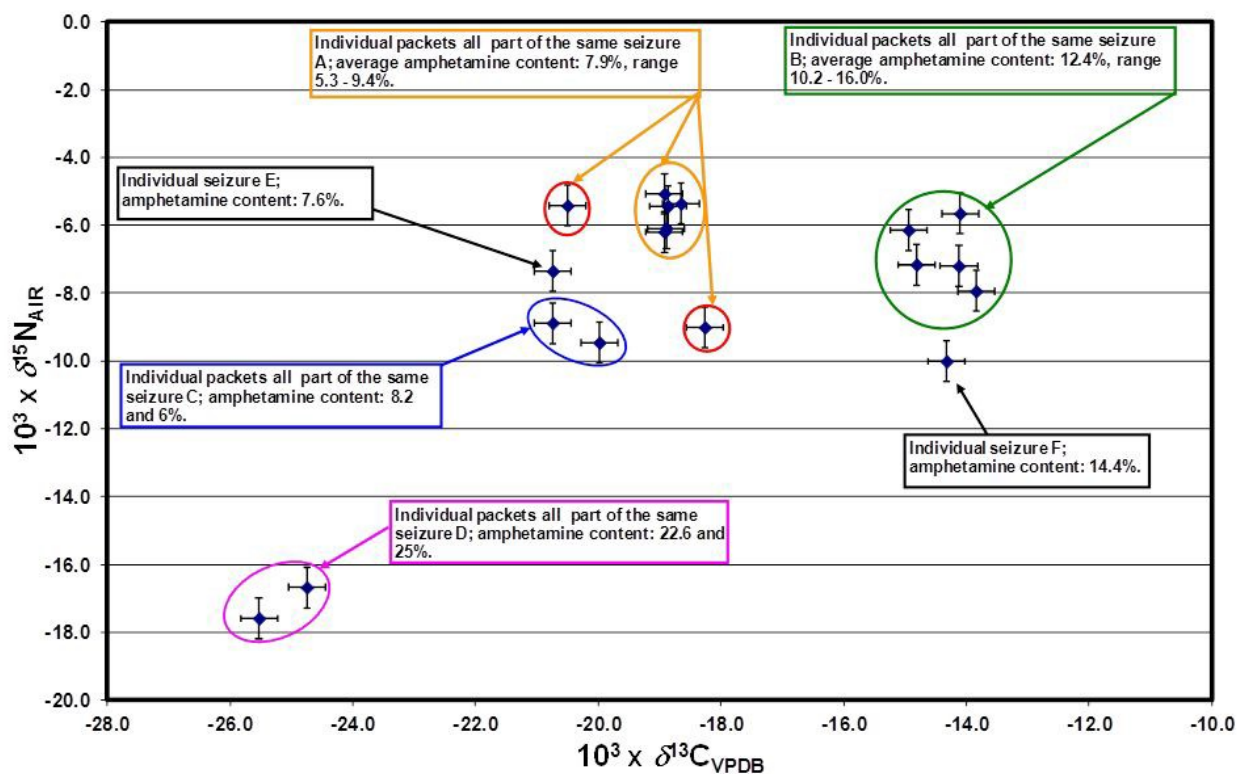


Figure 1: Plot of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ signatures of 18 amphetamine samples from six seizures. Reproduced with permission of John Wiley & Sons Ltd, from “Stable Isotope Forensics - Methods and Forensic Applications of Stable Isotope Analysis 2ed.”, Meier-Augenstein, W., © 2018.

2.2.1 Amphetamine

To test the potential usefulness of comparative multivariate stable isotope signatures a law enforcement agency gave us 18 amphetamine samples of known sample history to analyse in a blinded fashion. On the basis of two-dimensional [$\delta^{13}\text{C}$, $\delta^{15}\text{N}$] signatures these 18 samples were classed into eight different groups (Meier-Augenstein, W., 2018). In fact, the 18 samples were known to have come from six different sources. Two samples classed as not belonging to any other group had in fact come from the same source A as five other samples grouped together (Figure 1). Based on groupings our assessment had a 75 % hit rate while based on number of samples correctly grouped the hit rate was 88.89 %. Considering the range of amphetamine content within each group of samples, this example illustrates what powerful a tool stable isotope analysis can be for generating forensic intelligence. It is also worth noting simultaneous analysis of ^{13}C and ^{15}N abundance took only 4 hours and 40 minutes for all samples including standards

2.2.2 Heroin

In 2003, the Australian Federal Police arrested two people suspected to be involved in smuggling heroin into Australia. The two suspects were found to be in possession of 50 kg of heroin. On the day before their arrest the two suspects had been observed rendezvousing with the vessel Pong Su, a North Korean-owned freighter. Two days later, members of the Australian Special Operations Command boarded the Pong Su while in Australian territorial waters. In total, four men were convicted of importing almost 125 kg of heroin into Australia.

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3 Results of the forensic examination including stable isotope analysis of heroin samples taken
4 from 20 different kilogram packages were published three year later (Casale, Casale, Collins,
5 Morello, Cathapermal, & Panicker, 2006). While the $\delta^{13}\text{C}$ signature of heroin appeared to consistent
6 with that of heroin sample known to have come from South America, Southeast or Southwest Asia,
7 the $\delta^{13}\text{C}$ signature of morphine was consistent only with that of morphine of known South American
8 origin. Plotting morphine $\delta^{13}\text{C}$ values *versus* heroin $\delta^{13}\text{C}$ values clearly distinguished the Pong Su
9 samples from heroin samples of known to have originated from Mexico, South America, Southeast
10 or Southwest Asia. This finding was confirmed when $\delta^{13}\text{C}$ data of heroin and morphine were
11 combined with acetylcodeine/heroin abundance ratio values. It was concluded the heroin seized
12 from the Pong Su was found to be distinctly different from any heroin samples examined previously
13 which lead various drug enforcement agencies to speculate if the heroin seized from the Pong Su
14 might have come from a new region or might have been prepared by a process different from the
15 hitherto known.
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21 2.2.3 Benzocaine

22 While not an illicit narcotic drug itself, presumably due to its pharmacological properties benzocaine
23 has become the cutting agent of choice for cocaine. Due to its use as cutting agent for cocaine being
24 found in possession of large quantities of benzocaine is regarded as proof of being involved in a
25 criminal organisation or conspiracy to supply cocaine. In fact, in 2012 a criminal gang of 12 was
26 jailed for a combined total of 42 years and six months for importing at least 485 kg of benzocaine
27 into the UK.
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31 Unrelated to the aforementioned case our laboratory was contacted in 2011 by a law
32 enforcement agency asking us to aid their investigations by analysing various benzocaine seizures for
33 their stable isotope signatures to find out which if any showed signs of being related. So any level of
34 similarity or relationship between a particular pair of case samples could be statistically evaluated a
35 total of 51 control samples were collected and analysed in addition to the samples from two
36 separate cases. These 51 control samples included a pair of subsamples taken from the same parent
37 material used by a forensic services laboratory as analytical standard. Figure 2 shows the results of a
38 Principal Component Analysis (PCA) of case samples and a select group of controls based on their
39 mean $\delta^2\text{H}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values as four independent variables. Matching samples of cases A
40 and B are circled in red and orange respectively, while the known to be identical pair of control
41 samples of the analytical standard are circled in green. The PCA plot shown in Figure 2 is based on
42 results from work on seized samples of benzocaine reported previously (Meier-Augenstein, 2018).
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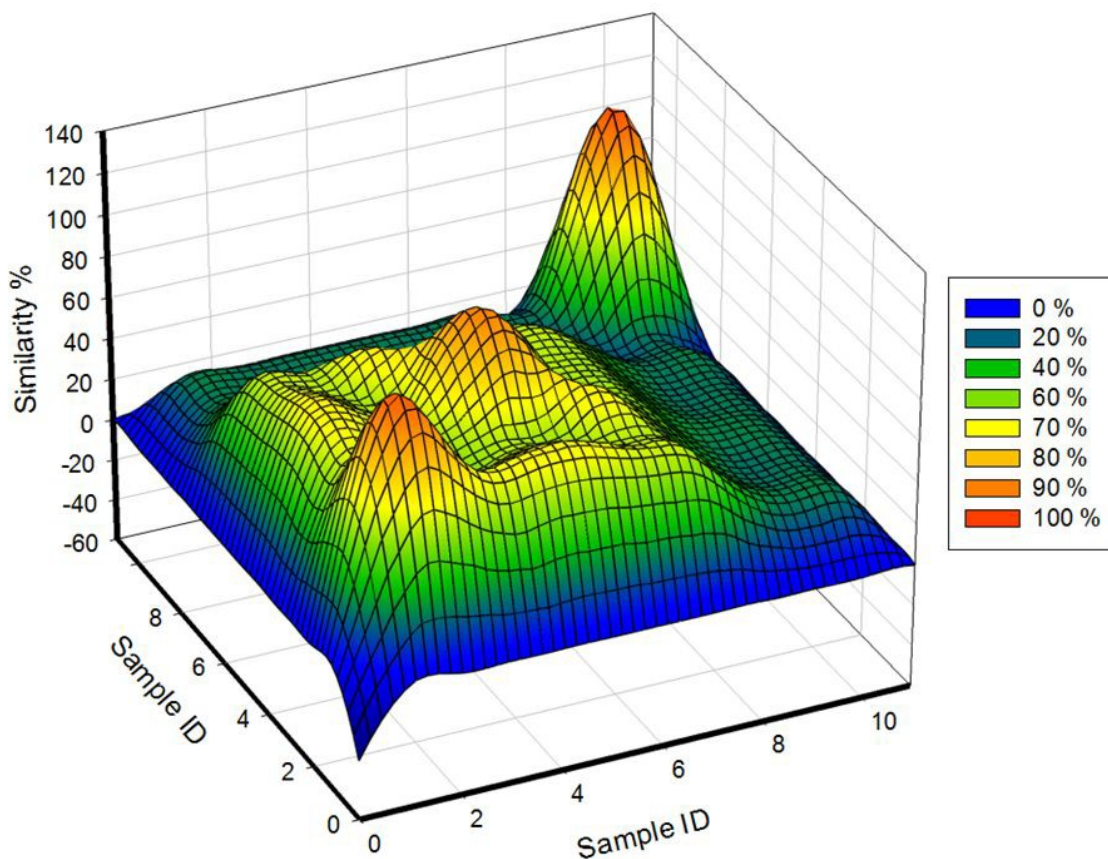


Figure 3: 3D topographical plot of similarity level between sample pairs based on HCA of 60 benzocaine sample (for sake of clarity results are shown for 10 samples only). Sample m is compared to sample n for all possible combinations meeting the condition $m \neq n$. Case samples #1 and #2 are similar at a level of 93 % but not related to case samples #4 and #5 which are similar at a level of 95 %. Known to be identical control samples #9 and #10 are 91 % similar.

As the examples presented in this section aptly illustrate, using multivariate stable isotope signatures for comparative analysis of drugs primarily with forensic intelligence purposes in mind does not mean data bases are not required. The amphetamine example mentioned in section 2.2.1 is already an indication of how limited data sets may obscure potentially valuable information. The strength of forensic intelligence based on statistical evaluation of results clearly depends on and will increase with the size of its underpinning data base. This is of particular importance when aiming to determine the level or likelihood ratio of two samples being similar or even a match with an acceptable level of statistical uncertainty. Further benefits of extensive data bases of multivariate stable isotope signatures will be discussed in section 3. Furthermore, should an investigative decision based on a presumptive comparative test be challenged in court, it will be essential to be able to refer to the data bases underpinning conclusions drawn from results of comparative analyses.

2.3 Human hair and nails

Comparing stable isotope signatures of human tissue such as scalp hair may serve one of two purposes; (i) to establish a chronology of a person's recent life history; (ii) to determine if an

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3 individual's life history is consistent with that of a person or persons whose life histories are known.
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5 The former would be based on intra-individual comparisons while the latter would be based on
6 inter-individual comparisons. Intra-individual comparative analysis aiming to establish a chronology
7 of changes in stable isotopic composition of hair and/or nails does not necessarily have to rely on a
8 data base if the objective is merely to look for evidence or absence of change that is potentially
9 related to a change in geographic location. A potential scenario for this may be law enforcement
10 agencies wishing to check on the veracity of a suspect's statement about their whereabouts during a
11 given time frame in cases of suspected terrorism involvement such as travel to and temporary
12 residence in a country known to harbour terrorist training camps (Meier-Augenstein & Kemp, 2012).
13 Should a suspect declare not having travelled and stayed abroad during a given time frame but the
14 pattern of changes in hair or nail stable isotope signatures suggests otherwise this would give law
15 enforcement agencies sufficient grounds to detain and investigate the suspect further. In short,
16 sequential analyses of human hair and/or nails, both of which can be sampled non-invasively, has
17 the potential of delivering valuable forensic intelligence.
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22 Making use of chronological changes in stable isotope signatures of sequentially analysed
23 hair or nail segments also has applications in investigations of murder or unexplained death in
24 particular if the identity of the deceased is not known and cannot be ascertained by traditional
25 means. Even in these circumstances person specific data bases are not required for interpretation of
26 results although what is required is sound knowledge of tissue specific correlation between isotopic
27 composition of water and isotopic composition of hair and nail (Bowen, Ehleringer, Chesson,
28 Thompson, Podlesak, & Cerling, 2009; Ehleringer, Bowen, Chesson, West, Podlesak, & Cerling, 2008;
29 Fraser & Meier-Augenstein, 2007; Fraser, Meier-Augenstein, & Kalin, 2006; Huelsemann, Lehn,
30 Schneiders, Jackson, Hill, Rossmann et al., 2015; Mancuso & Ehleringer, 2018; Mancuso & Ehleringer,
31 2019a; Mancuso & Ehleringer, 2019b; Nardoto, Silva, Kendall, Ehleringer, Chesson, Ferraz et al.,
32 2006; Reynard, Burt, Koon, & Tuross, 2016; Thompson, Chesson, Podlesak, Bowen, Cerling, &
33 Ehleringer, 2010). This kind of knowledge, which can only be gained from painstaking longitudinal
34 studies of both individuals and larger study populations, is essential for interpretation of results and
35 turning these results into a recent life history of a deceased not only for forensic intelligence
36 purposes to advance investigations but also to enhance information released for public appeals
37 (Meier-Augenstein, 2018). Several examples have been published during the last 10 years of how
38 stable isotope signatures or chronological profiles having aided with unravelling recent life history or
39 life trajectory of a Jane or John Doe. To the author's best knowledge in at least seven cases involving
40 unidentified victims of serious crime stable isotope signatures of human tissue have advanced their
41 investigation (Font, van der Peijl, van Leuwen, van Wetten, & Davies, 2015; Lehn, Rossmann, &
42 Graw, 2015; Meier-Augenstein, 2018; Meier-Augenstein & Fraser, 2008; Meier-Augenstein & Kemp,
43 2012; Remien, Adler, Chesson, Valenzuela, Ehleringer, & Cerling, 2014). In all seven cases, forensic
44 intelligence provided by stable isotope signatures was corroborated by police investigations. In two
45 of these cases forensic intelligence provided by stable isotope signatures was actually instrumental
46 in helping police to positively identify the victims (Meier-Augenstein & Fraser, 2008; Remien et al.,
47 2014).
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3 Geo-locating

Going from comparative analysis of individual samples to comparative analysis backed up by a substantial data base may seem like a small step. However, what represents a giant leap forward in terms of forensic intelligence provision are the kind of insights and understanding gained as well as the large data bases which result from the seemingly unimaginative and, hence, underfunded systematic and longitudinal research. For one, without large data bases meaningful statistical analysis and evaluation of results is nigh impossible. For another, large data bases enable us to look for statistically significant correlations and to create software models or algorithms based thereon that can be used to come up with a prognosis of expected characteristics for source or origin of a given sample. Probably one of the most impressive and far reaching outcomes of systematic and longitudinal research into stable isotope signatures of (bio)chemical compounds and materials is the development of a wide variety of matrix-specific isotope landscapes or “isoscapes” that illustrate in an impressive way the spatial (even temporal) variation in stable isotope abundance of compounds or materials of interest, such as drugs, human scalp hair or human tooth enamel as well as their ultimate precursors water and vegetation (Bowen & Revenaugh, 2003; Bowen & Wilkinson, 2002; Bowen, Winter, Spero, Zierenberg, Reeder, Cerling et al., 2005; Suits, Denning, Berry, Still, Kaduk, Miller et al., 2005).

Even though the focus of this review is on applications of stable isotope signatures in criminal case circumstance or in support of criminal case investigation and court proceedings, it is important to appreciate how relevant stable isotope signatures and statistical analysis of large data sets are for determining or excluding geographic provenance to the field of wildlife forensics (Bowen, Wassenaar, & Hobson, 2005; Hobson, Van Wilgenburg, Larson, & Wassenaar, 2009; Ziegler, Merker, Streit, Boner, & Jacob, 2016). Examples of such applications include the detection of illegally logged and wrongfully declared wood or violations of CITES regulations regarding trade of animals and animal products of protected species (Dormontt, Boner, Braun, Breulmann, Degen, Espinoza et al., 2015; van Schingen, Ziegler, Boner, Streit, Nguyen, Crook et al., 2016).

3.1 Natural drugs

Probably one of the most impressive results from the kind of painstaking systematic research are the cocaine isoscapes generated by the team at the DEA's Special Testing and Research Laboratory as part of their Cocaine Signature Program. They analysed cocaine isolated from 336 coca leaf samples of known provenance (Figure 4). Based on these isoscapes mapping spatial distribution of ^2H , ^{13}C , ^{15}N and ^{18}O isotope composition of cocaine and by using ArcGIS software it was possible to delineate the 19 major coca growing regions in South America (Malette, Casale, Jordan, Morello, & Beyer, 2016). Malette *et al.* (2016) also created alkaloid abundance landscapes based on abundances of the three minor alkaloids trimethoxycocaine, tropacocaine and truxillines relative to cocaine. Developing a predictive framework model on the basis of all geographically characterised samples it was possible to classify an unknown illicit sample of cocaine as originating from one of the 19 regions with an accuracy of approximately 96 %.

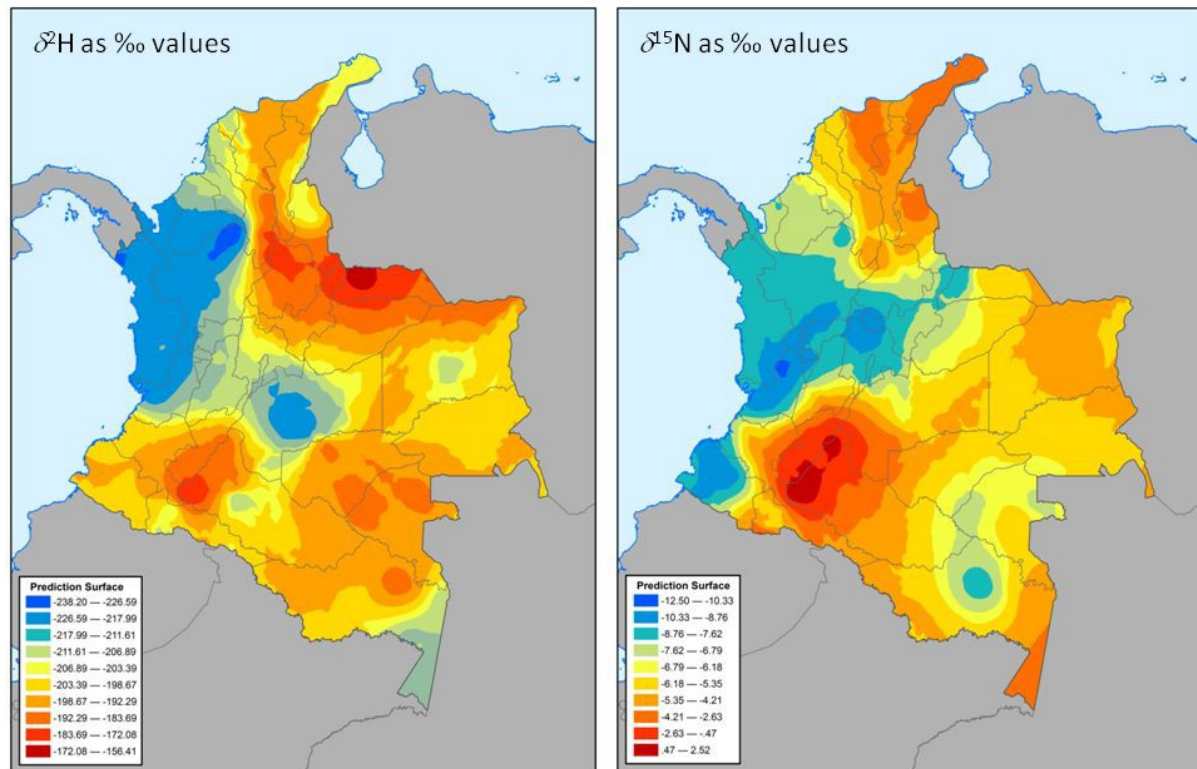


Figure 4: Isocapes of cocaine $\delta^2\text{H}$ (left) and $\delta^{15}\text{N}$ (right) values of Colombia based on 336 authentic samples prepared from coca leaves. Reproduced from Mallette *et al.* (2016) under Creative Commons License (<https://creativecommons.org/licenses/by/4.0/>).

Table 1: Mean stable isotope abundance value ranges^{a)} observed in authentic samples of cocaine from different South and Middle American coca-growing regions^{b)}.

	$\delta^2\text{H}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{18}\text{O}$
Chapare, Bolivia	-220.1	-34.5	-12.3	+15.1
Antioquia, Colombia	-228.7	-34.3	-7.6	+10.4
Arauca, Colombia	-167.8	-33.4	-7.4	+23.8
Caquetá, Columbia	-189.0	-34.3	-0.9	+18.6
Cauca, Colombia	-199.0	-33.3	-6.0	+16.3
Guaviare, Colombia	-191.9	-34.9	-5.2	+19.2
Vaupes, Colombia	-194.2	-35.7	-6.8	+17.5
Chiapas, Mexico	-178.3	-34.3	-12.9	+17.4
Cusco, Peru	-226.7	-33.7	-9.5	+18.3
HV, Peru	-219.0	-35.0	-8.1	+15.1
Lowest to highest	-228.7 to -167.8	-35.7 to -33.3	-12.9 to -0.9	+10.4 to +23.8
δ value range	60.9	2.4	12.0	13.4

^{a)} All δ values are presented as ‰ values.

^{b)} Table is based on data taken from Mallette *et al.* (2016) and Casale & Mallette (2016).

As part of the Cocaine Signature Program scientists at the DEA's Special Testing and Research Laboratory also carried out a series of systematic studies. As in the case of the aforementioned isoscape work, all these studies combined stable isotope signatures with minor alkaloid abundance profiles in support of multivariate chemometric analyses. Cocaine samples from other coca growing regions in South and Middle America were analysed and some results are shown in Table 1 (Casale & Mallette, 2016; Mallette, Casale, Jordan, Morello, & Beyer, 2016). No instance was found in which samples from two different regions exhibited indistinguishable stable isotope abundance values for hydrogen, carbon, nitrogen and oxygen simultaneously. Even if stable isotope abundance values for one or two elements were indistinguishable, the δ values of the other stable isotopes were different (cf. entries for Chapare, Bolivia and UHV, Peru in Table 1). Further systematic studies included determining the influence of extraction efficiency on the isotopic composition of the precipitated cocaine extract as well as identifying effects on cocaine signatures resulting from changes to the procedure of large scale cocaine hydrochloride processing (Mallette, Casale, Colley, Morello, & Jordan, 2018; Mallette, Casale, Jones, & Morello, 2017).

3.2 Human provenance

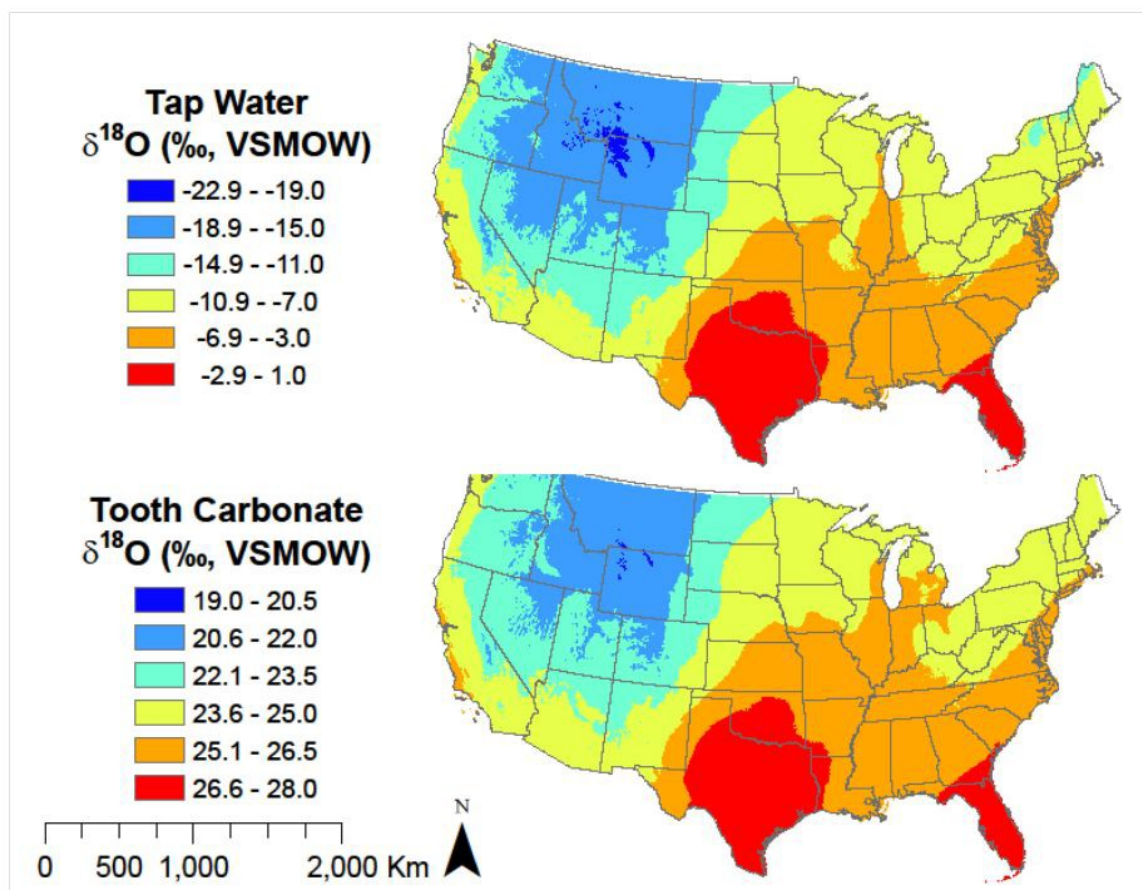


Figure 5: Isoscape of $\delta^{18}\text{O}$ values in tap water across the contiguous USA (top) and isoscape of $\delta^{18}\text{O}$ values in the carbonate fraction of tooth enamel bio-apatite throughout the USA (bottom). Reproduced with permission of John Wiley & Sons Ltd, from "Stable Isotope Forensics - Methods and Forensic Applications of Stable Isotope Analysis 2ed.", Meier-Augenstein, W., © 2018.

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3 Next to determining provenance of drugs, the application of stable isotope signatures of human
4 tissue in aid of forensic determination of human provenance is probably the other most impressive
5 example of geo-locating. Early studies on scalp hair and fingernails from more than 50 volunteers
6 from 12 different countries showed a strong correlation between tissue $\delta^2\text{H}$ values and $\delta^2\text{H}$ values of
7 source water (Fraser & Meier-Augenstein, 2007; Fraser, Meier-Augenstein, & Kalin, 2006). In 2008,
8 James Ehleringer and his research group published the first $\delta^2\text{H}$ and $\delta^{18}\text{O}$ isoscapes of human scalp
9 hair for the contiguous 48 states of the USA (Ehleringer et al., 2008). The models Ehleringer *et al.*
10 (2008) derived were underpinned by earlier work generating isoscapes of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in tap water
11 across the United States (Bowen, Ehleringer, Chesson, Stange, & Cerling, 2007). A similarly strong
12 correlation exists between $\delta^{18}\text{O}$ values of source water and that of bio-apatite, the mineral that
13 tooth enamel is comprised of and which is also present in bone. The $\delta^{18}\text{O}$ isoscape of the carbonate
14 fraction of tooth enamel bio-apatite shown in Figure 5 clearly illustrates the strength of correlation
15 that exists between this tissue and $\delta^{18}\text{O}$ values of source water (Ehleringer *et al.*, 2010). An in-depth
16 discussion of the correlation between stable isotope composition of human tissue and its links and
17 correlation with food and drink can be found in chapter III.5 of Meier-Augenstein's book (2018).

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22 Despite the strong correlation between ^2H or ^{18}O isotope composition of precursor pool
23 water and $\delta^2\text{H}$ or $\delta^{18}\text{O}$ values of product human tissue, the example shown in Figure 5 clearly
24 illustrates stable isotope signatures of human tissue are not able to point to one and only one point
25 as geographic origin of a person. One obvious reason for this is the fact that several disparate
26 locations may exhibit similar or near identical $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of water. The other reason is the
27 inter-individual variability of human physiology and metabolism which leads to a variability in stable
28 signatures of e.g. hair or fingernails from different individuals living in the same locale. In spite of
29 this seeming disadvantage, in the last 10 years, stable isotope signatures including chronological
30 stable isotope profiles of human tissue have been applied successfully to several criminal
31 investigations by providing forensic intelligence that helped to identify previously unidentified
32 victims of serious crime and thus advance police investigations (Font, van der Peijl, van Leuwen, van
33 Wetten, & Davies, 2015; Lehn, Rossmann, & Graw, 2015; Meier-Augenstein & Fraser, 2008; Meier-
34 Augenstein & Kemp, 2012; Remien et al., 2014). In times of increasing human mobility the task of
35 checking an unidentified body's dental records has become increasingly difficult. Stable isotope
36 signatures may help by narrowing down the number of locations or regions where a victim of crime
37 or natural disaster has originally come from thus providing focus to an investigation. To paraphrase
38 the needle in the haystack adage, stable isotope signatures of human tissue may not find the needle
39 in the haystack but they may reduce the haystack to a manageable size.

40 41 42 43 44 45 46 47 48 49 **4 Detecting linkage**

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51 Of course, one could argue geo-locating essentially links a piece of evidence such as drugs or human
52 tissue to one or at least a narrowed down number of locations. However, while a link between
53 sample and geo-location may be based on a highly significant correlation this does not denote a
54 direct causal link. The situation is different when it comes to the relationship between precursor
55 chemical or chemicals and product of a particular chemical synthetic process. Like the reaction rate
56 constant or better reaction rate coefficient k the isotopic fractionation factor α is a reaction specific
57 constant, i.e. depends on the nature of the reactants, temperature of the reaction and other
58 reaction parameters. In other words, if a compound A and reagent B react to form compound C
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from A and compound A does not react, i.e. does not get consumed quantitatively, the isotopic fraction factor α links the isotopic composition of compound A to that of compound C in a way that depends on the isotopic composition of compound A and the particular reactions conditions (cf. chapter 1.4 in (Meier-Augenstein, 2018)). Knowledge and insights of how a particular "recipe" or precursor source favoured by clandestine laboratories may be reflected in stable isotope signatures of the product will therefore make a valuable contribution to forensic intelligence. As importantly if not more so, being able to link a particular piece of evidence to a clandestine laboratory and the chemicals and reagents found therein will enable law enforcement agencies to conclusively link individual cells of a criminal or terrorist network. Stable isotope signatures may even provide the evidence that establishes links between different people and different crime scenes that might otherwise be difficult to substantiate.

4.1 Synthetic drugs

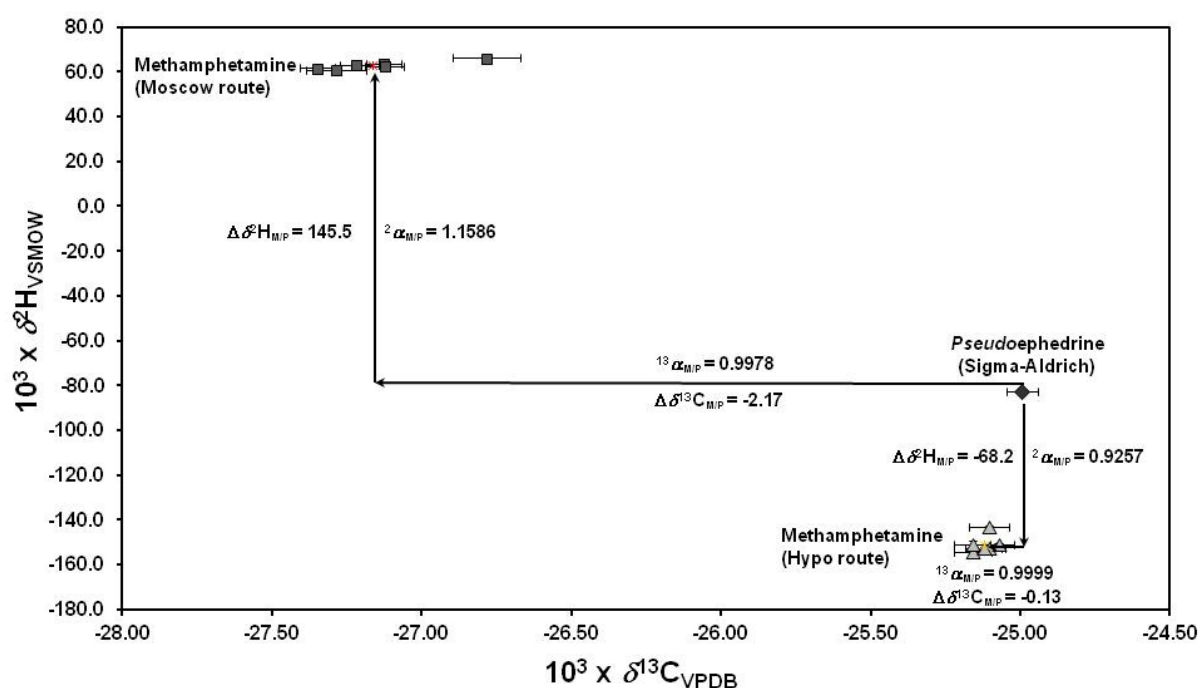


Figure 6: Bivariate isotope profile plot of $\delta^2\text{H}$ and corresponding $\delta^{13}\text{C}$ values of six methamphetamine batches per synthetic route each, synthesized from aliquots of the same precursor. Arrows and isotopic fractionation factors refer to the centroid positions of all batches per synthetic route. Reproduced with permission of John Wiley & Sons Ltd, from "Stable Isotope Forensics - Methods and Forensic Applications of Stable Isotope Analysis 2ed.", Meier-Augenstein, W., © 2018.

As early as 2002 it was suggested multivariate, i.e. multi-element stable isotope signatures of synthetic drugs such as methamphetamine or 3,4-methylenedioxy-N-methylamphetamine (MDMA) could yield information permitting to link seized powders or tablets to a common production batch and ultimately their common source (Carter, Titterton, Grant, & Sleeman, 2002). Probably the first kind of systematic study to be published on this subject reported the influence of three different reductive amination routes and corresponding different reaction conditions on multi-element stable isotope signatures of MDMA synthesised from piperonyl methyl ketone (PMK). To this end a large batch of PMK was synthesised from a single batch of safrole. Subsamples of PMK were used to synthesise MDMA*HCl by 3 different routes with 6 individual batches being prepared per synthetic

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3 route (Buchanan, NicDaeid, Meier-Augenstein, Kemp, Kerr, & Middleditch, 2008). While products
4 from both the Pt/H₂ and the NaBH₄ methods were indistinguishable on the basis of their $\delta^{15}\text{N}$ values,
5 they differed significantly from the $\delta^{15}\text{N}$ value of the product of the Al/Hg amalgam method.
6 However, products from the three methods could be distinguished in all instances on the basis of
7 their $\delta^2\text{H}$ and $\delta^{13}\text{C}$ values. Since then several studies have been published reporting results of
8 systematic investigations into how synthetic route affects stable isotope signatures of an illicit drug
9 made from identical subsamples of the same precursor. Synthesis of illicit drugs studied in this
10 regard include amphetamine (Collins, Salouros, Cawley, Robertson, Heagney, & Arenas-Queralto,
11 2010), methamphetamine (Collins, Cawley, Heagney, Kissane, Robertson, & Salouros, 2009;
12 NicDaeid, Jayamana, Kerr, Meier-Augenstein, & Kemp, 2013; Salouros, Collins, Cawley, & Longworth,
13 2012; Salouros, Sutton, Howes, Hibbert, & Collins, 2013), MDMA (Buchanan, Daeid, Kerr, Carter, &
14 Hill, 2010; Buchanan, Kerr, Meier-Augenstein, & Daeid, 2011; Schneiders, Holdermann, &
15 Dahlenburg, 2009) and cathinones such as mephedrone (Collins, Doddridge, & Salouros, 2016;
16 NicDaeid, Meier-Augenstein, Kemp, & Sutcliffe, 2012). The methamphetamine example shown in
17 Figure 6 illustrates what kind of differences in isotopic fractionation and corresponding differences
18 in stable isotope signature of the product can result from different synthetic routes, i.e. choice of
19 reagents and reaction conditions (NicDaeid, Jayamana, Kerr, Meier-Augenstein, & Kemp, 2013).

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26 It has to be pointed out most of the aforementioned studies on synthetic routes of illicit
27 drugs were carried out under controlled conditions. One tell-tale sign of well controlled reaction
28 conditions seems to be low variability of $\delta^2\text{H}$ values of key intermediates and final products while
29 not well controlled reaction condition seem to result in a much higher degree of variability. Seizures
30 of illegally trafficked 1-phenyl-2-propanone (P2P) showed a $\delta^2\text{H}$ value range of 109 ‰ while $\delta^2\text{H}$
31 values of P2P synthesised under controlled conditions for comparative purposes showed a range of
32 26 ‰ (Schneiders, Holdermann, & Dahlenburg, 2009). Similar observations were made by an
33 Australian forensic laboratory when comparing $\delta^2\text{H}$ values of amphetamine and a key intermediate,
34 1-phenyl-nitropropene, from a controlled synthesis and from samples seized at a clandestine
35 laboratory. However, despite the numerical differences $\delta^2\text{H}$ values from clandestine and controlled
36 sources were still similar enough to permit the conclusion they were made using the "nitrostyrene"
37 route (Collins et al., 2010). Other studies carried out under conditions aiming to mimic conditions in
38 small scale clandestine laboratories yielded results that showed a high level of variability in the
39 stable isotope data thus reflecting poorer control of reaction conditions. For example, deliberate
40 variation of reaction conditions such as reagent amount and/or reaction time during MDMA
41 synthesis by the Pt/H₂ method yielded products showing $\delta^2\text{H}$ and $\delta^{15}\text{N}$ value ranges of approx. 65
42 and 30 ‰ respectively compared to the narrow ranges of 2 and 1.1 ‰ respectively under controlled
43 conditions. However, meaningful discrimination by synthetic route could still be achieved by
44 chemometric analysis of a multivariate data set of stable isotope signatures in combination with
45 impurity profiles generated using the Collaborative Harmonisation of Methods for the Profiling of
46 Amphetamine Type Stimulants, i.e. the CHAMP profiling method (Buchanan, Kerr, Meier-Augenstein,
47 & Daeid, 2011).

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52 From an investigative perspective, a fair similarity between stable isotope signatures of
53 control samples and those of illicit drugs from a small-scale clandestine lab may still provide useful
54 forensic intelligence with regard to synthetic process and precursor chemicals used. Additional work
55 will however be required to conclusively prove to the satisfaction of a criminal court for linkage to
56 exist between drug and small-scale operations run by individuals at home or in a shed in their
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backyard. However, illicit drugs manufactured on a large scale by organized crime organizations are typically synthesized under almost “professional” conditions with large volumes of precursor chemicals being trafficked from one country into the countries where the actual drug is finally synthesised. In such cases, stable isotope signatures of drugs and precursor chemical and associated isotopic fractionation factors may well be instrumental in the investigation and conviction of individuals suspected to be involved in a criminal organisation by linking illicit drug dealer to illicit drug supplier to illicit drug lab to seized precursor chemicals.

4.2 Organic explosives

Stable isotope analysis of explosives is another area that has attracted interest by several law enforcement agencies or their associated forensic laboratories given the huge potential of stable isotope signatures to provide valuable forensic intelligence and possibly corroborative evidence in criminal trials (Aranda, Stern, Dietz, McCormick, Barrow, & Mothershead, 2011; Benson, Lennard, Maynard, Hill, Andrew, & Roux, 2009; Widory, Minet, & Barbe-Leborgne, 2009). In 2007, the first article looking at the potential of stable isotope signatures of an organic explosive in systematic way reported the results of a study evaluating bivariate stable isotope data of Semtex using a likelihood ratio framework (Pierrini, Doyle, Champod, Taroni, Wakelin, & Lock, 2007). Likelihood ratio using a multivariate Hotelling's T^2 -distance and a kernel density estimate yielded the best results. When Semtex came from the same source the proposition in favour of the prosecution was supported with no misleading evidence in favour of the defence proposition. However, in the converse case of the defence proposition being true there was misleading evidence in favour of the prosecution proposition at a rate of 5.5 %. The authors of this study felt this rate was still high and advised caution. However, the study was based on a bivariate data set of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. A data set comprising $\delta^2\text{H}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values might have offered greater discrimination. Furthermore, unbeknownst to the authors some of the samples analysed could have come from the same production batch. Knowing both sample source and sample history is of paramount importance for systematic studies aiming to create data bases and to analyse and evaluate those data.

Knowledge of the process or route used to synthesise a given explosive may point to sample source. Different manufacturers of industrial or military explosives may use different variations, i.e. different reaction conditions to carry out a given chemical reaction as is for example the case in the production of hexogen (aka RDX), a key constituent of C4 and Semtex. Hexogen (RDX) is basically the result of the nitration of hexamine which can be achieved by two different routes, named Woolwich and Bachmann process. It is therefore important to know the associated differences in both isotopic fractionation and product to by-product ratio. In 2008, the results of the first systematic study of its kind were published that determined isotopic fractionation factors $^{13}\alpha$ and $^{15}\alpha$ linking $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of product RDX made using the Woolwich process to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of precursor hexamine (Lock & Meier-Augenstein, 2008). A subsequent study carried out independently supported the findings of the 2008 study (Howa, Lott, Chesson, & Ehleringer, 2014). The study published in 2014 also investigated the influence of the reaction conditions of the Bachmann process on isotopic fractionation factors $^{13}\alpha$ and $^{15}\alpha$. While the shifts in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values going from precursor to product were +8.35 ‰ and -14.01 ‰ respectively for RDX made by the Woolwich process, in case of the Bachmann process the corresponding δ value shifts were +0.9 ‰ and -3.6 ‰ respectively.

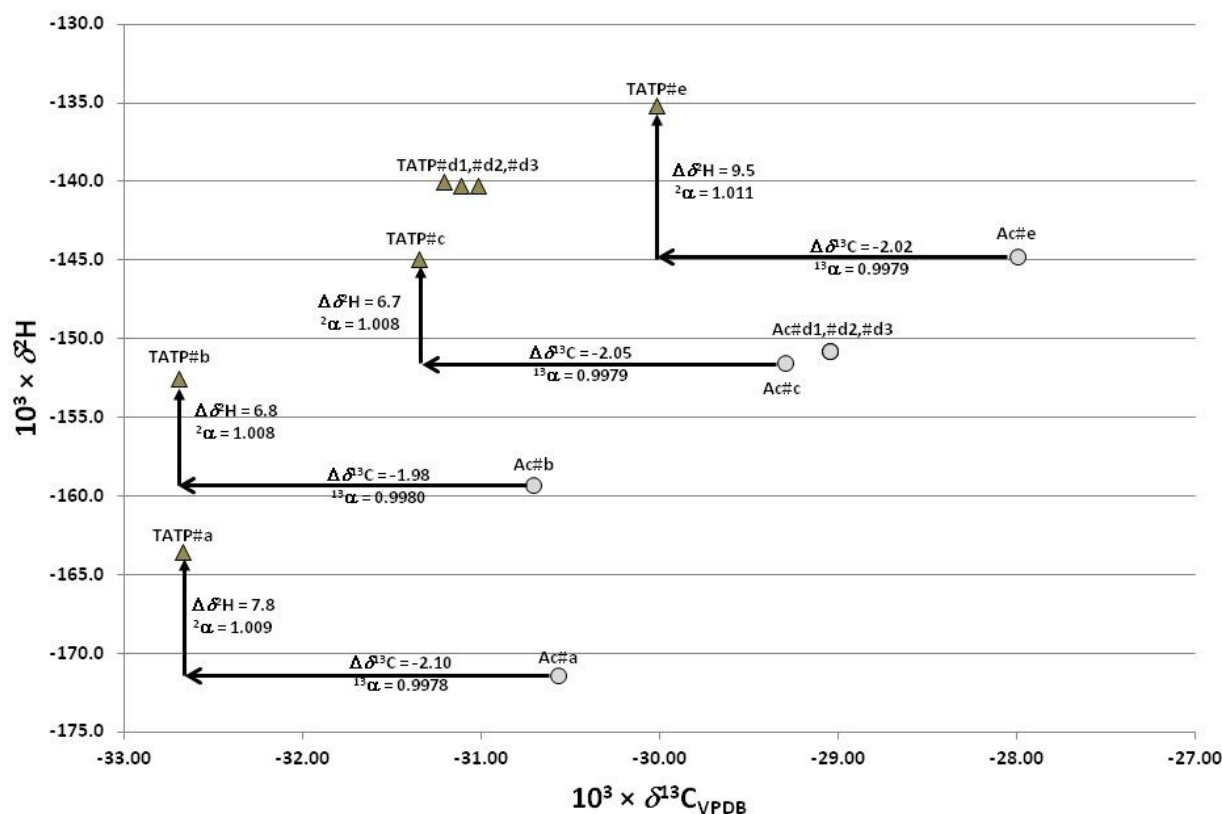


Figure 7: Bivariate isotope profile plot of $\delta^2\text{H}$ and corresponding $\delta^{13}\text{C}$ values of TATP made from acetone from different sources showing direction and magnitude of isotopic fractionation. Reproduced with permission of John Wiley & Sons Ltd, from "Stable Isotope Forensics - Methods and Forensic Applications of Stable Isotope Analysis 2ed.", Meier-Augenstein, W., © 2018.

Even in the case of improvised or home-made explosives such as triacetone peroxide (TATP) or hexamethylene triperoxide diamine (HMTD), knowledge of the synthetic process and its associated isotopic fractionation may yield valuable forensic intelligence helping to link precursor chemical to clandestine laboratory to explosive. Synthesising TATP or HMTD is deceptively easy though it does require some skill to control the conditions of the underlying reaction. However, owing to the ease with which precursor chemicals can be obtained and the relative simplicity of the synthetic process these explosives, in particular TATP have become a frequently used improvised explosive. An initial study carried out at the Forensics Centre of the Australian Federal Police analysed four batches of TATP synthesised under different reaction conditions. The products from the four different sets of conditions could be conclusively distinguished on the basis of their ^2H , ^{13}C and ^{18}O isotope signatures (Benson et al., 2009). Further systematic studies of the reaction producing TATP showed precursor acetone and product TATP were linked by fairly consistent isotopic fractionation factors $^2\alpha$ and $^{13}\alpha$ of +1.010 and 0.9979 respectively (Figure 7) even though reaction temperature could not be rigorously controlled and hydrogen peroxide concentrations varied between 25 and 35 % (Bezemer, Koeberg, van der Heijden, van Driel, Blaga, Bruinsma et al., 2016). Obviously, the $\delta^{13}\text{C}$ value of the product TATP depends entirely on the ^{13}C isotopic composition of the precursor acetone while the degree of ^{13}C isotopic fractionation will depend on reaction kinetics. The occurrence of a ^2H isotopic fraction is most likely owed to the formation of carbenium cations as key intermediates to which hydrogen peroxide anions are added (Meier-Augenstein, 2018). Results of a systematic study into the link between stable isotope signature of

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3 acetone and that of its product TATP support these findings (Howa, Barnette, Chesson, Lott, &
4 Ehleringer, 2018).

5 The data from the systematic studies into synthesis of explosives discussed above clearly
6 illustrate the great potential of forensic stable isotope analysis at near-natural abundance level
7 beyond the point of direct comparative isotope analysis of chemically identical materials, namely
8 enabling investigators to determine links between starting material and explosives product which
9 may be useful in scenarios where intelligence and ultimately evidence are required for establishing
10 links between individual cells of a terrorist network by linking chemicals seized at the premises of a
11 suspect to a clandestine laboratory and ultimately to an explosive device.
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18 **5 Concluding remarks**

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20 The above overview of the different ways in which stable isotope signatures can be applied in a
21 forensic context clearly demonstrates the great potential this technique brings to the investigation
22 of crime, even acts of terrorism. While this overview has focused on stable isotope signatures of
23 illicit drugs, explosives and human tissue, the potential and benefits of this technique should not be
24 overlooked when it comes to other types of evidence such as paint (Farmer, Meier-Augenstein, &
25 Lucy, 2009), paper (Jones, Benson, & Roux, 2013a; Jones, Benson, & Roux, 2013b; Jones, Benson, &
26 Roux, 2013c; Jones, Benson, & Roux, 2016; van Es, de Koeijer, & van der Peijl, 2009), plastic material
27 (Bezemer, Woortmeijer, Koeberg, Wiarda, Schoenmakers, & van Asten, 2018), sticky tape (Carter,
28 Grundy, Hill, Ronan, Titterton, & Sleeman, 2004; Dietz, Stern, Mehlretter, Parish, McLasky, &
29 Aranda, 2012; Nienaber, Cresswell, Carter, & Peter, 2018; Quirk, Bellerby, Carter, Thomas, & Hill,
30 2009), match sticks (Farmer, Curran, Lucy, Dauid, & Meier-Augenstein, 2009; Farmer, Ruffell, Meier-
31 Augenstein, Meneely, & Kalin, 2007) or toxins (Kreuzer, Horita, Moran, Tomkins, Janszen, & Carman,
32 2012; Kreuzer, West, & Ehleringer, 2013; Kreuzer-Martin, Lott, Dorigan, & Ehleringer, 2003; Tea,
33 Antheaume, & Zhang, 2012; Webb-Robertson, Kreuzer, Hart, Ehleringer, West, Gill et al., 2012).
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38 With regard to testability and peer review, stable isotope analytical techniques meet
39 conditions (1) and (2) of the four Daubert's principles as supported by the sheer number of
40 publications in peer reviewed scientific journals especially journals dedicated to forensic science and
41 the wealth of information contained therein. General acceptance of the underlying science (fourth
42 Daubert condition) is attested to by the wide spectrum of science areas applying stable isotope
43 analytical techniques to answer scientific questions which range from archaeology to zoology.
44 Meeting three of the four Daubert's conditions or principles may be considered sufficient for the
45 purpose of delivering forensic intelligence. However, for forensic intelligence to be at its most
46 powerful it is important to know the error rate of the technique used and of the answers it provides.
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48 Furthermore, results of a technique must also be controllable by known standards and accepted
49 operational guidelines. Besides, one should not overlook that forensic intelligence may turn into
50 forensic evidence the moment it has to be relied upon in court to support investigative decisions.
51 The third Daubert's condition on reliable evidence covers exactly these points of known error rate of
52 a technique and standard controlling its operation.
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57 It may not be widely known but the technique of stable isotope analysis is probably one of
58 the best or most controlled analytical techniques there is. International operational standards exist
59 regarding both stable isotope abundance measurement by isotope ratio mass spectrometry and
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3 reporting results therefore which are issued and regularly assessed and, if necessary, up-dated by
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5 the International Union of Pure and Applied Chemistry (IUPAC) and its Commission on Isotopic
6 Abundances and Atomic Weights (CIAAW) (Brand, Coplen, Vogel, Rosner, & Prohaska, 2014; Coplen,
7 2011). In 2018, the Forensic Isotope Ratio Mass Spectrometry (FIRMS) network published the
8 second edition of its Good Practice Guide for Isotope Ratio Mass Spectrometry which is freely
9 available from <http://www.forensic-isotopes.org/gpg.html>. Internationally accepted reference
10 materials of known isotopic composition are administered and supplied by internationally
11 recognised organisations such as the International Atomic Energy Agency (IAEA), the National
12 Institute of Standards and Technology (NIST) and the United States Geological Survey (USGS). Stable
13 isotopic abundance measurements meeting the aforementioned IUPAC standards are therefore
14 quality controlled and quality assured in a way that ensures results are both traceable to
15 internationally accepted points of reference and internationally comparable on a like-for-like basis
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17 (Coleman & Meier-Augenstein, 2014; Meier-Augenstein & Schimmelmann, 2019; Schimmelmann, Qi,
18 Coplen, Brand, Fong, Meier-Augenstein et al., 2016). Stable isotope based forensic intelligence is
19 therefore most certainly not some kind of lower standard of evidence. The difference between
20 forensic intelligence and evidence could be described succinctly in this way: intelligence is open -
21 evidence is closed; intelligence detects - evidence convicts (or exonerates).
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26 Stable isotope signatures of physical evidence clearly show great potential for intelligence led
27 policing. Stable isotope signatures of bulk materials are able to provide valuable information quickly
28 to the police, often within less than 24 hours thus helping to inform, even advance a time critical
29 criminal investigation. While stable isotope signatures on their own may already be able to provide
30 powerful information, their discriminatory and probative power will be increased significantly when
31 combined with information from independent analytical techniques such as GC/MS, ICP-MS, Raman
32 Spectroscopy or XRD to name but four. For example, combining compound specific isotopic profiles
33 from GC-IRMS with compound specific mass spectrometric profiles from GC/MS could potentially
34 become the technique of choice for illicit drug profiling. Using GC(/MS)-IRMS hybrid instruments
35 (Meier-Augenstein, 1995; Mudge, Meier-Augenstein, Eadsforth, & Deleo, 2010) permits the
36 recording of both stable isotope and mass spectrometric profiles from the same sample injected in
37 one analysis under identical gas chromatographic conditions which will satisfy requirements of
38 evidentiary reliability.
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43 Last but not least, this overview should also serve as an example and timely reminder to
44 governments, government departments and research funding organisations that robust forensic
45 science requires equally robust funding. Without funding it is impossible to carry out the kind of
46 systematic and longitudinal research required (1) to enable meaningful and robust data analysis by
47 Bayesian, chemometric or other statistical approaches; (2) to create the algorithms and isotopic
48 landscapes required to detect and identify patterns; (3) to generate multilayered maps or data plots
49 that can be interrogated for correlations or links using ArcGIS, all of which could change the way law
50 enforcement agencies target, investigate and prevent crime
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