Determining the nature of exposure in athlete doping violations using isotopes

John D. Howa

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with Vinod Nair, Jenna Goodrum, Christine Doman, Matthew Scott Morrison, & Geoffrey Miller

Isotope ratio mass spectrometry is the gold standard test for detection of doping with testosterone. This test is based on the difference in the $^{13}C/^{12}C$ ratio in synthetic testosterone and endogenous steroids produced in humans. When exogenous (synthetic) testosterone is administered, the carbon isotope signatures of urinary testosterone and its metabolites are altered by incorporation of the administered preparation. The extent of change depends on the isotope ratio of the administered drug as well as dosage and the route of administration. To determine the window of detection of various preparations, controlled administration studies have been performed and data from these will be presented. Based on these studies, it may be possible to determine the nature of the exposure that led to a doping violation in an athlete sample. A relevant case study will be presented.

Evaluation of the endogenous or exogenous origin of testosterone in children by carbon isotope analysis: Report of two cases by the Drug Control Centre

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with Richard Harries, Daria Lipska, Cheyanne Pierre, Karl Chandler, Rodrigo Alexandre Aguilera, & Kim Wolff

Precocious puberty and children's virilization may occur as a consequence of health conditions like tumors, congenital adrenal hyperplasia, McCune-Albright syndrome and testotoxicosis, inducing high testosterone (T) levels in boys and girls. Cases of sexual precocity and elevated T in children's blood have also been reported when associated with skin-to-skin transfer of exogenous T from fathers undergoing topical T replacement therapy. The Drug Control Centre (DCC) – King's College London was asked by two different healthcare institutions to analyse children's urinary samples and ascertain the origin of T present in them. Both had a history of T replacement therapy using gel formulations in the families. Therefore, this study aimed to ascertain the origin of urinary T and metabolites in clinical samples from two child patients by GC/C/IRMS analysis.

The endogenous steroid profile was determined by gas chromatography - tandem mass spectrometry (GC-MS/MS). In brief: Each sample (2 mL) was hydrolysed using β-glucuronidase and then underwent solid-phase extraction. Afterwards, the residue was derivatized with enol reagent. Deuterium-labelled reference materials were used as internal standards. The samples were analysed on an Agilent J&W Scientific HP-1 column. The exogenous origin of the steroids was determined by gas chromatography - combustion - isotope ratio mass spectrometry (GC/C/IRMS). In brief: Each sample sub-aliquot (5 mL) underwent liquid-liquid extraction. The aqueous layer was hydrolised and then extracted using solid-phase extraction. Samples were then purified using high performance liquid chromatography with a fraction collector, and the final fractions were analysed on an Agilent J&W Scientific DB-35MS column.

The concentrations (ng/mL) of the endogenous steroids in each sample were determined, as well as the T/E ratio. The results obtained for two samples from patient A (A1 and A2) and one sample from patient B were:

A1: T = 0.9; Epitestosterone (E) = 0.7; Androsterone (Andro) = 45.1; Etiocholanolone (Etio) = 17.5; 5α-Androstane-3α,17β-diol (5α-Adiol) = 1.7; 5β-Androstane-3α,17β-diol (5β-Adiol) = 2.1; Pregnanediol (PD) = 9.5; 11-Keto-etiocholanolone (11K) = 52.0; T/E ratio = 1.33.

A2: T = 1.0; E = 0.4; Andro = 38.3; Etio = 16.8; 5α-Adiol = 1.9; 5β-Adiol = 0.4; PD = 6.6; 11K = 63.1; T/E ratio = 2.54.

B: T = 2.1; E = 0.3; Andro = 64.1; Etio = 40.9; 5α-Adiol = 4.4; 5β-Adiol = 2.8; PD = 20.8; 11K = 104.8; T/E ratio = 7.61.

Both A1 and A2 samples presented self-consistent results.

The T/E ratio is the most sensitive marker of the misuse of T or a precursor. In children aged about 12 years the average T/E ratio of all boys with typical development whereas has been reported as 1.37 ± 0.9 versus 2.03 ± 1.4 in girls with typical development. In the doping control field, whose criteria are based on adult individuals, a sample presenting T/E greater than 4.0 is flagged as a suspicious sample. Andro and Etio were analysed by GC/C/IRMS as target compounds in samples A1 and B, and 11K was used as endogenous reference compound.

The δ^{13}C values obtained in each sample were: A1: Andro = -28.3 ‰; Etio = -26.9 ‰; 11K = -23.6 ‰. B: Andro = -27-3 ‰; Etio = -26.9 ‰; 11K = -23.0 ‰.

The calculated δ^{13}C values against 11K as ERC in each sample were: A1: Andro = 4.7 ‰; Etio = 3.4 ‰, and B: Andro = 4.3 ‰; Etio = 3.9 ‰.

Applying WADA criteria to determine the origin of T it was possible to conclude that exogenous T was present in both patients. As a conclusion, GC/C/IRMS proved to be a valuable tool in the forensic field to provide direct evidence of the synthetic origin of T present in children.

Identification of synthetic urine by analysis of natural carbon and nitrogen isotope ratios

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with Laura Franke, Dirk Wissenbach, Gregor Fußhöller, & Mario Thevis

Manipulation of urine samples for drug testing or doping control analysis is a well-known problem, including dilution of urine, chemical adulteration or substitution by other liquids. Different analytical strategies have been developed to identify diluted or adulterated urine samples, as well as the identification of urine samples substituted with so-called synthetic urine. Synthetic urines may contain different substances, including creatinine, uric acid and urea and show specific gravities and pH similar to natural human urine samples. Methods evolved within the last years to identify synthetic urine samples with routine analytical methods based either on the identification of substance solely present in synthetic, but not natural urine, or on the identification of the absence of typically present urinary biomolecules.

In doping control analysis, the absence of natural occurring endogenous steroids in a urine sample is strong evidence for a manipulated e.g. highly diluted or substituted sample. However, the absence of endogenous steroids is no evidence for the substitution of the original sample by synthetic urine.

The present study investigated carbon and nitrogen stable isotope ratios of authentic and synthetic urine specimens. Two different urine specimen sets were analyzed. For set A, 43 adherence urine specimens were randomly intermixed in a double-blind manner with 8 synthetic urine products purchased from the Austrian/German market. Set B consisted of mixtures of urine specimens with the 8 synthetic urine specimens in different ratios.

Using EA-IRMS the synthetic urine specimens could clearly be differentiated from the human urine samples as well as mixtures of 10:90 and 50:50 (synthetic urine:human urine) offering a new method for the identification of adulterated and/or substituted urine samples in doping control analysis and forensic investigations.

Advancing isotope ratio measurements of trace metals and metalloids in the environment: Results of MetroPOEM project in the context of environmental forensics

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National Measurement Laboratory, LGC ltd

with Betul Engin, Philip Dunn, Dirk Arnold, Emma Braysher, Oktay Cankur, Lukas Flierl, Johanna Irrgeher, Shaun Lancaster, Aaron Lehnert, Johanna Noireaux, Marcus Oelze, Seena Prem Pranav, Jochen Vogl, Daniel Proefrock, Sonia North, Olaf Rienitz, Janine Eberhardt, Axel Pramann, Ben Russell, Christian Alexander Schöpke, & Tea Zuliani

The European Partnership on Metrology is an ambitious research and innovation programme co-funded by the Member States of EURAMET, the Regional Metrology Organisation of Europe, and the European Union. The Partnership project “Metrology for the harmonisation of measurements of environmental pollutants in Europe” (21GRD09, MetroPOEM) was funded under the Green Deal Call of the programme. Specific objectives of MetroPOEM include the development of new and improved methods of isotope amount ratio measurements of stable and radioactive elements, the development of reference materials and comparison of the performance of different instrumental techniques, including inductively coupled plasma mass spectrometry (ICP-MS) and thermal ionisation mass spectrometry (TIMS).

In this presentation, the potential of different ICP-MS based mass spectrometric techniques to resolve natural variations in the isotopic composition of Li, B, Ni, Cr, Cd, Pb and U of samples from different environmental compartments that can be used for source apportionment will be discussed. The significance of SI traceable measurements for ensuring accuracy and comparability of isotope ratio data between laboratories and over time will be highlighted. As an aid in obtaining SI traceable results, new and improved calibration approaches for the determination of absolute isotope ratios by normalisation to an internal standard will be presented.

Exploring the potential of amino acid δ^2H analysis as a forensic tool to identify region-of-origin

Emma A. Elliott Smith

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with Christy J. Mancuso (presenting), Hannah Vander Zanden, & Seth D. Newsome

Hydrogen isotope (δ^2H) analysis of keratinaceous bulk tissues has been used as a forensic tool to reconstruct an individual's travel history or determine their region-of-origin. Recent controlled feeding experiments, ranging from bacteria to mammals, hint at the potential of amino acid (AA) δ^2H analysis to trace geographic origins. Here, we expand on a published dataset of human scalp hair collected from known origin individuals (n=152) across the United States to evaluate how well AA δ^2H values reflect local geography. Our ongoing project collects metadata on age, sex, travel history, dietary preferences, and δ^2H of local tap water. δ^2H values of non-essential amino acids varied by up to 200‰ among individuals, likely reflecting a greater contribution of water in their synthesis and the potential for these dispensable compounds to exchange hydrogen with body water. Alanine δ^2H values were correlated with local tap water δ^2H, indicating that this molecule is a key tracker of regional water and precipitation inputs. In contrast, δ^2H values of the essential amino acids isoleucine, leucine, valine, and phenylalanine were relatively invariant (SD > 11‰) and uncorrelated with tap water, likely reflecting a consistent isotopic signature of nationally distributed food sources tied to integrated supply chains. Multivariate analysis of δ^2H values from both essential and non-essential amino acids enabled successful geographic classification of individuals into one of five regions of origin (midwest, northeast, northwest, south, and southeastern United States), with overall accuracy ranging from 70% to 90%. However, classification accuracy was consistently higher for individuals from rural areas compared to those from urban areas, suggesting more regionally specific isotopic inputs in rural diets. Our findings suggest that amino acid δ^2H analysis could help improve geolocation models for human and wildlife forensics by simultaneously providing information about dietary and drinking water sources of hydrogen to keratinaceous tissues.

A comparative study of isotope ratios in human bones and hair from a known contemporary skeletal collection

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Vanderbilt University, Department of Anthropology

with Gregory E. Berg & Lesley A. Chesson

Stable isotope ratio analysis of hair and bone can provide information about an individual's diet. By measuring δ^{13}C and δ^{15}N values, individuals can be sorted by the type of plants and the amount of protein in their diet. Dietary habits that are typical of specific cultures and geographies can provide a useful sorting strategy for large numbers of individuals and commingled assemblages of human skeletal remains. Isotope analysis is being used with growing frequency in forensic contexts, and there is an increasing need to demonstrate that the methods that have been commonly applied in archaeological studies are applicable to contemporary populations. While there are many published datasets of isotope delta (δ) values of human hair from contemporary populations around the world, there are far less data available for human bone. This study uses human bone and hair samples from the UTK Donated Skeletal Collection at the University of Tennessee, Knoxville, a contemporary skeletal collection of known individuals, to compare the isotope ratios obtained from bone collagen and hair of the same individual. It builds upon the work of O'Connell and colleagues (2001) and further explores the potential of directly comparing data for modern humans (from hair keratin) to archaeological data (from bone collagen). This presentation will discuss a dataset of δ^{13}C and δ^{15}N values from a United States population consuming a relatively similar "supermarket" diet, providing a deeper understanding of the relationship between the isotope δ values from hair and bone collagen within the same individual with the goal of making widely available isotopic data from hair more useful for predicting and estimating bone collagen values.

Reference:
O'Connell, T. C., Hedges, R. E., Healey, M. A., & Simpson, A. H. R. (2001). Isotopic comparison of hair, nail and bone: modern analyses. Journal of Archaeological Science, 28(11), 1247-1255.

Isotopic signatures of insects from decomposing carcasses

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with Aline M. do Prado, Patricia J. Thyssen, & Luiz A. Martinelli (presenting)

Decomposing carcasses undergo physical, chemical, and biological transformations. Necrophagous insects, feeding on decomposing tissues, reflect these changes through their carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopic signatures. We investigated the variation in δ^{13}C and δ^{15}N signatures of necrophagous insects throughout decomposition. Stillborn piglets were placed on the ground in an urban-rural area in central-eastern São Paulo state, Brazil. The carcasses were isolated by cages (macrofauna exclusion) and spaced 3 meters apart. Tissues and insects were subsampled from the carcasses until the dry remains stage. Insects were identified in families and morphotypes. Samples were analyzed using IRMS. We analyzed the δ^{13}C and δ^{15}N signatures (response variables) as a function of decomposition time (predictor variable). Each piglet was considered a factor. Preliminary results focused on Diptera order. Sarcophagidae and Calliphoridae represented more than 90% of these insects. The δ^{13}C variation during the decomposing of carcasses tends to be low, and necrophagous insects reflect the δ^{13}C of their sources (carcasses) plus isotopic fractionation (0-1‰). Thus, we expected similar values between carcasses and insects. There were no variations in δ^{13}C of tissues along the decomposition processes, but insects enriched in δ^{13}C over time. Initially, eggs and larvae showed δ^{13}C values similar to the environment (~-20‰), but over time, reflected the carcass values (~-14‰). On the other hand, the δ^{15}N in carcasses tends to increase over the decomposition process. Also, the δ^{15}N is a trophic level indicator, with an enrichment of 2-4‰ per trophic level. Over time, we observed a δ^{15}N enrichment of the tissues and insects (plus ~3‰). These findings validate the δ^{13}C and δ^{15}N as quantitative tools in forensic contexts, offering insights into nutrient dynamics during decomposition. Based on the isotopic patterns in insects, we expect in the future to be able to associate δ^{13}C and δ^{15}N variation with postmortem interval estimation.

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Intracortical radiocarbon ($F^{14}C$) and stable isotope variation in human femora

Karolina Cameron-Werens

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with Rick Schulting, Rachel Wood, Kevin Salesse, Christophe Snoeck, Amy Styring, Jamie Cameron, David Chivall, Kassim Javaid, & Andrzej Weber

Human bone, commonly used in archaeology and forensics for radiocarbon dating (^{14}C) and stable isotope analysis, has differential intra-skeletal turnover rates. Diet in adults is conventionally investigated using a single bone collagen measurement, resulting in a low temporal resolution averaging of the biochemical data, interpreted as a long-term 'adult' diet. Single-measurement bulk samples thus inevitably mask potential isotopic shifts and important dietary variation that may have occurred over time in life. The aim of this study is to define relative differences in human cortical bone turnover rates, based on 25 femoral bone collagen $F^{14}C$ (fraction modern) dates and associated carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope measurements. The data came from five modern donors who lived through the ^{14}C 'bomb spike' caused by nuclear testing in the mid-20th century, enabling precise bomb curve calibration of the ^{14}C measurements. Our results demonstrate a sequential variation in biochemical composition of the collagen in femoral cortex. From the $F^{14}C$ variation in modern femora, we infer a novel sampling approach for isotope analysis. In archaeology and forensics this will allow the recreation of chronological dietary and mobility history in adult life at an unprecedented resolution and will improve ^{14}C dating precision in forensic contexts.

Optimized static headspace sampling method for carbon isotope analysis of methanol and ethanol in water matrix

Mario Tuthorn

Thermo Fisher Scientific

with María de Castro, Ana I. Cabañero, & Vanessa Peiro

Analyzing alcohols in water matrix using gas chromatography is challenging due to the large aqueous component content in the sample. Water in the sample shortens the lifespan of GC columns and requires frequent maintenance of the injector and column. This can complicate the analysis of isotope signatures in wine and spirits by GC-C-IRMS, for an example, where $\delta^{13}C$ measurements of ethanol are a useful indicator for verifying product authenticity and preventing fraud.

Here we present an optimized method for carbon isotope analysis of volatile alcohols in water matrix by GC-IRMS that eliminates the need for prior alcohol isolation, thus simplifying sample preparation and speeding up analysis. The methodology is based on Static Headspace Sampling (SHS) injection of methanol and ethanol via a split/splitless injector. The isotopic data obtained by SHS GC-IRMS analysis of the alcohols standard mixes demonstrate excellent precision and accuracy, including high sensitivity and low detection limits. In addition to standards, we compare SHS GC-IRMS $\delta^{13}C$ data of wine and spirits samples and samples distillates with previously acquired $\delta^{13}C$ of the same samples analyzed by the OIV official method (based on the EA-IRMS analysis).

The newly developed methodology substantially reduces the total sample preparation time, simplifies the analytical setup and makes routine SHS GC-IRMS analysis of ethanol in wines and spirits feasible.

The next generation LC-IRMS for honey authenticity investigation

Niel Williams

Thermo Fisher Scientific

with Mario Tuthorn (presenting), Daniel Felsmann, Nils Stoebener, & Qiong Li

LC-IRMS is a state-of-the-art technique for the detection of low-level adulteration of authentic honey with sugar syrups. The new Thermo Scientific™ LC IsoLink™ II IRMS System delivers a reliable, robust and efficient solution for high precision honey fraud detection using compound specific carbon isotope signature of individual sugars.

The next generation LC IsoLink II Conversion Interface is now fully integrated in the innovative Thermo Scientific™ Vanquish™ LC platform. The modular pull-out design is saving space and allows easy accessibility of all system parts without de-stacking for routine maintenance. The new cartridge-based oxidation reactor minimizes flow path blockage and significantly enhances system uptime and productivity. Full LC IsoLink II IRMS System operation is driven by the Thermo Scientific™ Qtegra™ ISDS Software that features complete integration with Chromeleon™ Chromatography Data System Software capabilities. Single software platform setup simplifies workflows, saves time and minimizes errors.

To assess long-term stability, system robustness and data reproducibility, the LC IsoLink II IRMS System has been operated in analytical food testing laboratories for over 2 years, allowing thorough system evaluation and optimization. We report data demonstrating excellent precision and reproducibility of $\delta^{13}C$ values for measurements of a laboratory honey standard and commercial honey samples.

POSTER SESSION

Fingernail stable isotope diagenesis: Effects of taphonomy and storage on carbon, nitrogen, oxygen, hydrogen, and sulphur

Melanie M. Beasley

Purdue University

Forensic stable isotope analysis is most beneficial to forensic investigations of unknown human remains in cases where standard methods of identification are unsuccessful. Stable isotope analysis capitalizes on the use of stoichiometric models to understand how elements move through ecological systems, creating unique "fingerprints" of elements incorporated into human biological tissues. Despite successful applications in forensic anthropology of stable isotope analysis, there are still basic research questions about alteration of biological samples after death that need to be addressed for wider adoption by the medicolegal community. The University of Tennessee Forensic Anthropology Center's documented donors provide a unique opportunity for such research because of the biological tissues curated, varied scenarios for decomposition (buried vs surface), and standardized documentation of life history movement. This project looks at the postmortem alteration of δ^{2}H, δ^{18}O, δ^{13}C, δ^{15}N, and δ^{34}S in fingernails in three scenarios: 1) differences in storage containers over 2 years of sample storage, 2) change in isotope values over 3 years for 26 individuals, and 3) a 2-year longitudinal study of 12 individuals. In all three experiments, carbon and nitrogen remain interpretively consistent, while alteration in oxygen, hydrogen, and sulphur values are significant. This is of forensic importance because diagenesis of elemental signatures recorded in physical evidence may impact interpretations of region-of-origin predictions if isotope ratios are altered by taphonomy and long-term storage.

POSTER SESSION

A journey through accreditation of a stable isotope analysis method for modern human collagen

Thuan H. Chau

SNA International, working under contract with the Defense POW/MIA Accounting Agency

with Lesley A. Chesson, Daniel L. Johnson, & Gregory E. Berg

There is rapidly growing interest in and application of stable isotope analysis (SIA) in the fields of forensic science and anthropology. The isotope delta values of nitrogen (δ^{15}N), carbon (δ^{13}C), and sulfur (δ^{34}S) from bone collagen provide insights into the dietary habits and provenance of individuals.[1,2,3] Recent advancement with instrumentation allows for more routine simultaneous measurement of δ^{15}N, δ^{13}C, and δ^{34}S values from a small amount of collagen.[4] The Defense POW/MIA Accounting Agency (DPAA) Laboratory has adapted and optimized a published SIA method for triple-gas (NCS) measurement of bone collagen to support human identification efforts. Following the 10-point method validation plan described by Dunn et al.,[5] SIA at the DPAA was accredited in Fall 2020, falling within the DPAA Laboratory's Scope of Work as "Geographic Profiling." This is the first SIA facility in the United States accredited for forensic investigation of human remains. Here, we describe the stakeholder requirements and source, scope, and protocol for the SIA method used at the DPAA to measure δ^{15}N, δ^{13}C, and δ^{34}S values of bone collagen. We also describe the data specifically collected during the validation process to assess the working range, precision, accuracy (bias), and ruggedness/robustness of the method; precision and accuracy data were used to calculate measurement uncertainty for the method. Finally, we discuss external validation and fitness-for-purpose of the method. The 10-point method validation plan of Dunn et al. is a valuable guide for any laboratory adding new or modifying existing SIA methods to their analytical toolbox.

References:

1. V. Raoult, A. A. Phillips, J. Nelson, Y. Niella, C. Skinner, M. B. Tilcock, P. J. Burke, P. Szpak, W. R. James, C. Harrod, Why aquatic scientists should use sulfur stable isotope ratios (δ^{34}S) more often. Chemosphere 355 (2024), 141816. https://doi.org/10.1016/j.chemosphere.2024.141816.

2. J. Kveiborg, A. Uzunidis, L. Klassen, F. Klimscha, N. N. Johannsen, U. L. Rasmussen, M. P. Richards, J. Svenning, Paleodiet reconstructions and human utilization of middle Holocene *Equus ferus* in northwest Europe, Palaeogeography, Palaeoclimatology, Palaeoecology 649 (2024), 112334. https://doi.org/10.1016/j.palaeo.2024.112334

3. A. Amaro, B. T. Fuller, A. Fornaciari, V. Giuffra, J. Olsen, M. A. Mannino, Increased C4 cultivation and consumption as an impetus for population growth at Badia Pozzeveri, Italy (11th–13th Centuries CE), Journal of Archaeological Science: Reports 57 (2024) 104620. https://doi.org/10.1016/j.jasrep.2024.104620

4. K.L. Sayle, C.R. Brodie, G.T. Cook, W.D. Hamilton, Sequential measurement of δ^{15}N, δ^{13}C and δ^{34}S values in archaeological bone collagen at the Scottish Universities Environmental Research Centre (SUERC): A new analytical frontier, Rapid Communications in Mass Spectrometry 33 (2019) 1258–1266. https://doi.org/10.1002/rcm.8462.

5. P.J.H. Dunn, H. Salouros, J.F. Carter, S.P. Doyle, Forensic application of stable isotope delta values: Proposed minimum requirements for method validation, Rapid Communications in Mass Spectrometry 31 (2017) 1476–1480. https://doi.org/10.1002/rcm.7925.

POSTER SESSION

IsoRep – Lessons learned from stable isotope database development

Michellei Fisher

SNA International, working under contract with the Defense POW/MIA Accounting Agency

with Amelia J. Edwards & Lesley A. Chesson

The Defense POW/MIA Accounting Agency (DPAA) Laboratory is the largest skeletal identification laboratory in the world whose mission is to provide the fullest possible accounting of missing and unidentified United States' military personnel. Over the last few years, the DPAA Isotope Program has expanded significantly, highlighting the need for a custom, scalable data repository capable of meeting forensic standards. The research surrounding development of the Isotope Repository (IsoRep) and lessons learned are presented here. IsoRep is a relational database that stores sample information and test results from isotope sample preparation and analysis. IsoRep records were compiled from internal data, external provider data, and research initiatives, covering 7+ years. Due to the complex nature of DPAA casework, a data flow chart was first mapped to isotope laboratory processes to pinpoint key data sources, identify important information, and solicit input on the use of IsoRep. IsoRep was developed on the Microsoft Power Platform, a collection of cloud services typically included with government, business, and educational Microsoft 365 licenses. Isotope data are uploaded directly from controlled Excel templates into SharePoint, which acts as the data storage location and primary access control. IsoRep data are automatically extracted daily into Power BI, where they are transformed and loaded into the relational model for exploration and visualization. IsoRep is innovative because it utilizes a low-code platform for managing isotope data and is being validated under forensic testing laboratory standards. IsoRep facilitates isotope data retrieval and supports research initiatives, laboratory task coordination, and quality control monitoring in the DPAA Isotope Program. This presentation serves as a reference for the design, validation, and management of stable isotope data, offering valuable insights for other laboratories with unique workflows aiming to implement scalable, standardized isotope data solutions.

POSTER SESSION

SIRMS Lab – A multidisciplinary research facility at the University of Southampton

Bastian Hambach

Ocean and Earth Science, University of Southampton

with M.A. Wilding & P.A. Wilson

The Stable Isotope Mass Spectrometry Laboratory (SIRMS Lab) at the University of Southampton is a state-of-the-art research, teaching and analytical facility dedicated to high-precision light stable isotope analysis across a diverse range of scientific disciplines. Located within the Ocean and Earth Science department of the University of Southampton at the National Oceanography Centre Southampton, Waterfront Campus, the lab supports academic research, external collaboration and commercial sample analysis, whilst offering expert guidance on sample preparation, analysis, and data interpretation.

Equipped with several advanced gas source isotope ratio mass spectrometers (IRMS) and peripheral devices operating in both dual inlet and continuous flow modes, the SIRMS lab specializes in analysing light stable isotopes such as δ^{2}H, δ^{13}C, δ^{15}N, δ^{18}O, and δ^{34}S across a wide array of organic and inorganic materials. This analytical capability supports research in environmental sciences, oceanography, geology, archaeology, biology, and climate science.

Researchers benefit from tailored support by dedicated personnel for sample preparation, instrument maintenance, method development, quality assurance and sample analysis, ensuring high-quality data and robust interpretations.

The lab staff is actively involved in training and mentoring students and postdoctoral researchers, fostering the next generation of scientists in stable isotope techniques.

Here we present an overview of the lab and its capabilities, introduce our new instrumentation with examples of recent research applications, demonstrating its role in advancing scientific understanding across multiple fields.

POSTER SESSION

The effect of humic substances on isotope delta values of bone collagen

Lyndi Low

SNA International, working under contract with the Defense POW/MIA Accounting Agency

with Lesley A. Chesson

Bone collagen is an analytical substrate for stable isotope analysis that is commonly used in archaeological and forensic studies. However, interactions with burial contaminants, particularly humic substances, could alter its chemical and isotopic compositions, potentially affecting the reliability of results. This study examines whether the presence of humic acids needs to be controlled prior to stable isotope analysis of bone collagen extracted from modern forensic remains. Sixty paired bone samples from casework at the Defense POW/MIA Accounting Agency (DPAA) Laboratory were used in this study. For each pair, a sample that underwent either one or two rounds of sodium hydroxide (NaOH) treatment to remove humic acids during the collagen extraction process was compared to a sample that was not treated with NaOH. The δ^{15}N and δ^{13}C values of extracted bone collagen were measured via EA-IRMS, and differences (Δ) between treated and untreated samples were compared to RID_{prep} limits. Using a paired samples t-test, no statistically significant difference was found in δ^{13}C values between treated and untreated samples ($p = 0.8149$, $t = 0.2352$), while δ^{15}N values showed a statistically significant difference ($p = 0.0001$, $t = 10.2656$). However, all Δ for δ^{15}N values were <0.75‰, the RID_{prep} limit, indicating the statistical difference was not practically meaningful. In contrast, 18% (11 of 60 pairs) of Δ for δ^{13}C values exceeded the RID_{prep} limit of 0.43‰, suggesting that skipping NaOH treatment had a meaningful impact. Additionally, atomic C:N ratios differed significantly between treated and untreated samples ($p = 0.0013$, $t = 3.4042$), further highlighting the effect of humic substance contamination. The findings support the inclusion of NaOH treatment to remove humic substances during the bone collagen extraction process prior to stable isotope analysis, particularly for measurement of δ^{13}C values.

POSTER SESSION

High precision Isotope Ratio-Orbitrap-MS – A new tool for vanillin and caffeine authentication

Christy Mancuso

Thermo Fisher Scientific

with Mario Tuthorn, Nils Kuhlbusch, Andreas Hilkert, & Dieter Juchelka

Vanillin and caffeine are widely used in food and beverage products. They can be sourced from natural or synthetic materials, through various production processes. The source of such compounds is of great interest as it carries cost implication for the final product, resulting in great demand for analytical techniques for source differentiation. Until recently, sector field isotope ratio MS and NMR have been the main tools used for these analyses. Here we demonstrate the ability of soft ionization-Orbitrap IRMS to provide high precision isotope ratio determination of intact caffeine and vanillin molecular ions with an option for fragmentation. This offers a unique opportunity for the analysis of intramolecular isotopic information like multiple heavy isotope substitutions (isotope clumping) as well as site specific isotopic information.

In this study we will highlight benefits of using Thermo Scientific™ Orbitrap Exploris™ Isotope Solutions for simultaneous C, H, N and O isotope ratios analysis from intact caffeine and vanillin molecular ions, specifically focusing on sample introduction and efficiency of analysis. Different methodologies and referencing strategies are evaluated to increase accuracy and precision of isotope ratio data. Orbitrap Exploris Isotope Solutions results are compared and benchmarked against international reference materials and the results obtained by sector field MS. Preliminary results of vanillin and caffeine analysis show accuracies and precisions down to ~0.5 ‰ for carbon, 2 ‰ for oxygen, 10 ‰ for hydrogen and 1 ‰ for nitrogen isotope ratios.

POSTER SESSION

National Network of Forensic Isotopes (RENIF): Strengthening forensic sciences through integrated isotopic analysis

Gabriela Bielefeld Nardoto

Universidade de Brasilia

with Rodrigo Ribeiro Mayrink, Cristina Barazetti Barbieri, & Fabio Augusto Salvador

The Brazilian National Network of Forensic Isotopes (RENIF) was founded in 2019 during the Interforensics Conference, bringing together researchers, faculty, students, and forensic experts to consolidate the use of isotopic analysis in forensic sciences in Brazil. Formalized as a scientific association, RENIF connects universities, forensic institutions, and third-sector partners to develop robust methodologies for provenance analysis across multiple contexts — including wildlife, cultural heritage, food fraud, drugs, and environmental crimes. Among its achievements are unprecedented isoscapes for the Brazilian territory, that are being applied in real cases, peer-reviewed articles, forensic reports, technical manuals, and training activities. A major milestone is the publication of the book “Isótopos Forenses” (sold by Millennium Editora, 2022), organized by RENIF’s board and collaboratively written by academic and forensic members. The book has become a national reference for professionals, researchers, and civil society. RENIF has also played a crucial role in catalyzing isotopic laboratory services in Brazil for forensic studies and promoting interlaboratory tests, enhancing analytical quality and reliability. The methodologies organized and fostered by RENIF are now routinely applied at the National Forensic Isotopes Laboratory of the Brazilian Federal Police (LANIF), contributing to complex crime investigations. The network actively supports training highly qualified professionals, bridging academic research and forensic practice. Thus, RENIF stands out as a collaborative, multidisciplinary, and innovative network, expanding isotopic analysis as a strategic tool for advancing forensic sciences in Brazil. More information: www.renifbrasil.org

POSTER SESSION

The timing of demineralization in the bone collagen extraction process for isotopic analysis

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with Tiffany B. Fracchia, Shirly Montero, Gwyneth W. Gordon, & Lesley A. Chesson

Bone collagen extraction for isotopic analysis is a lengthy process with demineralization being the most time-consuming step. Mineral removal from bone involves multiple rounds of acid treatment over several days to several weeks. To understand the rate of mineral removal, a study at Arizona State University (ASU) measured concentrations of calcium and phosphorus removed from a fresh cow rib during demineralization and found the average duration of demineralization was 12.0 ± 3.2 days (n = 5). Based on this study, we hypothesized that the collagen extraction process used at the Defense POW/MIA Accounting Agency (DPAA) Laboratory for human bone samples would follow a similar demineralization timeline regardless of the chemical preservation status. The DPAA Laboratory is a high throughput facility that utilizes isotopic analysis of bone collagen to assist with identification of unknown remains of missing military personnel. Bone samples nominated by Laboratory staff for isotopic analysis are generally grouped into two categories based on prior chemical preservation: either untreated or treated (i.e., chemically preserved prior to burial in a cemetery). We compared the duration of demineralization for untreated and treated bone samples prepared for isotopic analysis at the DPAA Laboratory since 2019 and assessed the impact of chemical preservation on the timing of bone collagen extraction. As expected, untreated bone (mean = 12.7 ± 4.5 days, n = 1,029) aligns with the duration of demineralization determined by the previous evaluation of the fresh cow rib. In contrast, treated bone has an extended duration of demineralization (mean = 17.0 ± 7.1 days, n = 2,882). Therefore, the timing of bone collagen extraction from treated bone takes approximately one more week than untreated bone.

POSTER SESSION

The use of sulfur isotopes ($\delta^{34}S$) in the identification and investigation of unidentified skeletal human remains in British Columbia Canada.

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with Laura Yazedjian & Michael Richards

This study explores the emerging use of sulfur stable isotopes ($\delta^{34}S$) of human tissues as a geographic indicator, complimenting the more established oxygen and strontium systems. Sulfur isotope ratios are predominantly influenced by distance from the coast, and local soil chemistry. Similar to oxygen and strontium isotope ratios, it is necessary to have a bioavailable baseline (isoscape) to interpret the $\delta^{34}S$ values of human tissues as geographic indicators.

This poster presents our pilot $\delta^{34}S$ isoscape based on foliar $\delta^{34}S$ isotope ratio across southern British Columbia, Canada. Furthermore, we demonstrate the potential applications of $\delta^{34}S$ values to the investigation of unidentified human remains (in collaboration with the British Columbia Coroners Service) as indicators of their geographical origin.

Unlike strontium and oxygen isotope ratios, which are commonly measured in enamel and reflect childhood origins, sulfur isotopes can be measured in tissues that turn over during adulthood, providing insight into geographic location of the years preceding the individual's death.

A stable foundation for the use of tooth enamel oxygen isotopes in human identification

Gabriel J. Bowen

University of Utah, Department of Geology and Geophysics

with Kirsten A. Verostick, Chris Stantis, Stephannie Covarrubias, Thomas A. Delgado, Lesley A. Chesson, & Gregory E. Berg

Stable isotope ratios in human body tissues vary geographically and are potentially useful in reconstructing life history and identifying unknown decedents. The oxygen isotope composition (δ^{18}O value) of tooth enamel carbonate is a promising target for this work due to its expected correlation with drinking water δ^{18}O values, but we lack a large, well-documented, standardized database of human tooth enamel δ^{18}O values as a foundation for forensic applications. Building such a database covering the contiguous USA is the goal of Project FIND-EM, a collaboration between the academic research community and the U.S. Defense POW/MIA Accounting Agency. Third molars have been collected from human donors through acquisitions from existing tooth and skeletal collections and contributions by living participants. Life history information spanning the period of third molar formation was also obtained, and samples were prepared and analyzed using standardized, experimentally supported laboratory methods.

Sample collection has been successful but also highlights challenges inherent in developing large-scale human tissue isotope databases. Contributions from living participants have varied between geographic regions, producing low sample numbers in some regions (particularly those with low population density) and large numbers in locations served by highly engaged dental industry partners. Despite this, representative coverage has been achieved across much of the contiguous USA. Measured δ^{18}O values of tooth enamel carbonate show strong, coherent spatial patterns that correlate well with known patterns of variation in tap water δ^{18}O values. Isoscape models for enamel carbonate developed using two methods (kriging and linear regression against tap water) show that the majority (~75%) of the observed variation in tooth enamel carbonate δ^{18}O values is predictable based on geographic location. However, both methods reveal substantial (1σ = 1‰) variability among different individuals living within the same area. This implies that non-spatial factors such as individual behavior and physiology or environmental heterogeneity contribute appreciably to enamel δ^{18}O values, limiting the precision of isoscape predictions to ±1‰ (1σ) placing bounds on the resolution of geographic assignments made using enamel carbonate δ^{18}O measurements.

Improving inter-laboratory comparability of tooth enamel carbonate stable isotope analysis ($\delta^{13}C$, $\delta^{18}O$)

Chris Stantis

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with Lesley A. Chesson, Daniel L. Johnson, Thuan H. Chau, Kirsten A. Verostick, Gregory E. Berg, & Gabriel J. Bowen

Carbon and oxygen isotope ratios of human tooth enamel carbonate can be used to aid in forensic identification efforts. Despite a long history of applications, tooth enamel is prepared and analyzed using a remarkably broad range of protocols, and this methodological heterogeneity raises questions about the comparability of isotopic data across studies. We report a systematic comparison of isotope delta (δ) values for 10 "modern" faunal teeth (obtained from field settings like what might be encountered during casework) measured in two different laboratories. Our comparison included pairs of enamel powder samples that were chemically pretreated using commonly adopted protocols and samples that received no pretreatment. We also evaluated δ values generated with and without (1) standardizing the reaction temperature used for carbonate acidification and (2) baking the samples to remove moisture before analysis. The results showed that δ values from the two laboratories were systematically different when samples were chemically pretreated, but that differences were smaller or negligible for untreated samples. Standardization of acid reaction temperature and baking also improved comparability, especially for $\delta^{18}O$ values. We suggest that the widely adopted practice of chemical pretreatment of tooth enamel samples is largely unnecessary and may compromise the comparability of isotopic data between studies.

Intra-individual and within-tooth isotopic variability: Implications for forensic identification

Kirsten Verostick

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with Chris Stantis, Thomas A. Delgado, & Gabriel J. Bowen

Project FIND-EM is developing standardized methods and a national dataset of tooth enamel carbonate $\delta^{18}O$ values to support human identification in the United States. A central aim is to ensure methodological rigor and promote consistency in forensic isotopic applications. This study supports that goal by examining intra-individual and intra-tooth isotopic variation and assessing its potential impact on geographic provenancing.

Many factors can contribute to intra- and inter-tooth isotopic variability, including differences in tooth development and mineralization timing, dietary shifts, physiological changes, and life history events such as relocation. Although previous studies have explored isotopic variation between tooth types or within individual teeth, to our knowledge none have examined isotopic differences within the same tooth type across or within arcades.

To investigate intra-individual variability, oxygen and carbon isotope data were collected from third molar enamel (n=164). Measurements were compared within individual teeth and between teeth from both the same and different arcades. We hypothesized that isotopic variability would be greater between teeth than within a single tooth, and greater between arcades than within the same arcade. Intra-tooth $\delta^{18}O$ differences averaged 0.26‰ (range = 0.00–1.23‰, n=328). Inter-tooth differences averaged 0.29‰ (range = 0.02–0.71‰, n=32) within arcades, and 0.23‰ (range = 0.00–2.60‰, n=132) between arcades. For $\delta^{13}C$, intra-tooth variation averaged 0.14‰ (range = 0.00–1.32‰), inter-tooth variation within arcades averaged 0.21‰ (range = 0.01–0.71‰), and inter-tooth variation between arcades averaged 0.16‰ (range = 0.00–2.01‰). Statistical tests (F-test, paired t-test, Wilcoxon) showed no significant differences for any of the comparisons for either isotope system. These results refute our hypotheses and suggest minimal intra-individual variation in third molar enamel carbonate isotope chemistry, supporting the interchangeable use of same-type teeth in forensic isotope analysis.

Accounting for human mobility in bulk enamel isotope analysis

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with Kirsten A. Verostick, Stephannie Covarrubias, Chris Stantis, Benjamin Rivera, Michiko A. Zaharias, Sagarika Banerjee, & Gabriel J. Bowen

The analysis of oxygen isotope "delta" values ($\delta^{18}O$) in human dental enamel is a well-established tool for estimating the geographic origin of unidentified human remains, providing insights into residency during childhood and adolescence. This method relies on the correlation between the isotopic composition of local drinking water and enamel formed by individuals residing in that region. However, efforts to accurately interpret $\delta^{18}O$ are complicated for mobile individuals that imbibe water from multiple residences. As of 2022, internal migration within the United States reached over 8 million people (U.S. Census Bureau 2024) underscoring the need for interpretive frameworks that consider the effect of movement during enamel development.

This study explores the impact of residential mobility on bulk enamel carbonate $\delta^{18}O$ values using data from Project FIND-EM, which has developed a reference database of isotope delta values from third molars for individuals with well-documented residential histories during molar formation (ages 7 to 15; AlQahtani et al. 2010). Most individuals (n=43) had two documented residences during this period with fewer living in three (n=6) or four (n=2) locations. Results show a clear detachment between the observed $\delta^{18}O$ values of mobile individuals and the expected $\delta^{18}O$ water values for their latest residence during molar formation (R^2=0.33). Averaging the $\delta^{18}O$ values across each residence improves the correlation (R^2=0.48), but a time-weighted average considering the duration of each residence yields the strongest relationship (R^2=0.55). The number of residences does not substantially impact this trend with similar correlations observed between individuals with two or three residences. These findings demonstrate the potential to identify and model residential mobility from a single bulk sample, laying the groundwork for future applications in forensic contexts where the $\delta^{18}O$ value measured for a tooth from an unknown decedent may be compared to residential patterns reported in a missing person record.

How to validate a tooth enamel carbonate carbon & oxygen isotope analysis method: A tale of unexpected challenges and important lessons learned

Daniel L. Johnson

SNA International, working under contract with the Defense POW/MIA Accounting Agency

with Thuan H. Chau, Lesley A. Chesson, & Gregory E. Berg

Carbonate $\delta^{13}C$ and $\delta^{18}O$ values from tooth enamel bioapatite are increasingly used in forensic contexts as an indicator of provenance. Here, we describe work completed at the Defense POW/MIA Accounting Agency (DPAA) Laboratory to validate a GasBench-isotope ratio mass spectrometry (GasBench-IRMS) method for measurement of these values (Chesson et al., 2019; Duhr & Hilkert, 2004; Revesz et al., 2001). Following a 10-point method validation plan (Dunn et al., 2017), we first describe the stakeholder requirements (Point 1) and protocol (2). We subsequently describe the data collected to determine the working range (3); precision (4); accuracy, or bias (5); and ruggedness/robustness (6) of the method. Matrix variations (Point 7) were minimized by ensuring adherence to the Principle of Identical Treatment (Carter & Fry, 2013) and by measuring equivalent masses of carbon and oxygen among materials. Data from the precision and accuracy determinations were used to calculate measurement uncertainty (8), which was 0.27‰ for $\delta^{13}C$ values and 0.77‰ for $\delta^{18}O$ values. Finally, we discuss external validation (9) of the method via reanalysis of human enamel samples initially measured by another laboratory. We found that the addition of a baking step (24-hour, 80 °C) prior to analysis reduced mean differences in isotope delta values to levels below measurement uncertainty, demonstrating the fitness-for-purpose of the method (Point 10). Based on this validation experience, we share a key takeaway that was unanticipated when we started method validation procedures: sample baking prior to GasBench-IRMS analysis appears to be an important step to attain inter-laboratory comparability of enamel carbonate $\delta^{13}C$ and $\delta^{18}O$ values. Additional considerations we discuss (Johnson et al., 2024) include the chemical treatment (or lack thereof) of enamel prior to analysis, the use of a low (< 30 °C) acid digestion temperature, and the incorporation of matrix-matched materials for quality assurance purposes.

References:

Carter, J. F., and B. Fry. “Ensuring the reliability of stable isotope ratio data—beyond the principle of identical treatment.” Analytical and Bioanalytical Chemistry 405 (2013): 2799-2814.

Chesson, L. A., M. W. Kenyhercz, L. A. Regan, and G. E. Berg. “Addressing data comparability in the creation of combined data sets of bioapatite carbon and oxygen isotopic compositions.” Archaeometry 61, no. 5 (2019): 1193-1206.

Duhr, A., and A. Hilkert. “$\delta^{18}O$ and $\delta^{13}C$ determination of carbonates Using Thermo Scientific GasBench II.” Thermo Fisher Scientific Application Note 30050 (2004).

Dunn, P. J. H., H. Salouros, J. F. Carter, and S. P. Doyle. “Forensic application of stable isotope delta values: Proposed minimum requirements for method validation.” Rapid Communications in Mass Spectrometry 31, no. 17 (2017): 1476-1480.

Johnson, D. L., T. H. Chau, and L. A. Chesson. “The oxygen isotopic composition of tooth enamel carbonate: A review of measurement methods & forensic applications.” TrAC Trends in Analytical Chemistry (2024): 118084.

Revesz, K. M., J. M. Landwehr, and J. E. Keybl. “Measurement of $\delta^{13}C$ and $\delta^{18}O$ isotopic ratios of $CaCO_3$ by a Thermoquest Finnigan GasBench II Delta Plus XL continuous flow isotope ratio mass spectrometer with application to Devils Hole Core DH-11 calcite.” USGS Open-File Report No. 2001-257. (2001).

Using non-exchangeable hydrogen isotope analysis to trace wildlife origins across aquatic and terrestrial boundaries

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with Maela Merlet, Sandra Iurino, & Christian C. Voigt

Hydrogen isotope analysis (δ^2H) has become a versatile and increasingly essential tool in wildlife forensics. Recent improvements in sample pre-treatment and calibration have allowed researchers to obtain more accurate and reproducible δ^2H values, especially in materials like keratin. In terrestrial wildlife studies, δ^2H values from tissues like fur, feathers, claws, and muscle reflect the isotopic composition of the water and food consumed, which correlates with regional precipitation patterns, and ultimately identify the geographic origin of bats, birds and fish. For example, δ^2H analysis of bat fur revealed that nearly 30% of noctule bats killed by wind turbines in France were long-distance migrants, highlighting the cross-border impact of energy infrastructure on vulnerable populations and the need for mitigation strategies. Other study in North America using δ^2H measurements helped confirm the Greenlandic origin of a Greylag Goose shot in New Brunswick in 2007—providing the first probable wild-origin record of the species on the continent and supporting evidence of increasing transatlantic vagrancy of waterfowl from the northeastern Atlantic. These δ^2H analysis is also valuable in aquatic ecosystems. In a study of Lake Winnipeg fish, the findings underscore the utility of hydrogen isotopes for tracing fish provenance and movements, while emphasizing the need to control for body size and environmental water δ^2H when applying the method across aquatic species. However, studying δ^2H in animals that traverse both aquatic and terrestrial environments is particularly challenging due to complex hydrogen exchange dynamics, differing water sources, and variable metabolic and physiological processes that influence tissue isotope values. Therefore, as a first step toward understanding δ^2H dynamics across ecosystem boundaries for animal provenance, we investigated a bat species that feed on both aquatic and terrestrial environments to assess how dual habitat use influences hydrogen isotope composition in its tissues and its applicability to assignment-to-origin modelling.

Advancing wildlife forensics: Multi-isotopic evidence to combat animal trafficking in Brazil

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Illegal wildlife trade remains a persistent threat to biodiversity, motivating the Brazilian Federal Police to adopt innovative scientific tools for forensic investigations. Since 2020, more than 20 cases involving trafficked wild animals have been supported by stable isotope analyses to clarify the provenance and captivity conditions of seized specimens. These investigations have encompassed diverse taxa—including birds, mammals, and reptiles—with emblematic examples such as Lear's Macaw (*Anodorhynchus leari*), the Golden Lion Tamarin (*Leontopithecus rosalia*), Amazonian turtles (*Podocnemis erythrocephala*), and green anacondas (*Eunectes murinus*).

This multi-isotopic approach integrates carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), hydrogen ($\delta^{2}H$), and oxygen ($\delta^{18}O$) signatures obtained from tissues such as feathers, hair, carapaces, and scales. Tissues are sectioned at the tip and base to capture temporal variations: the tip represents the oldest portion formed when the animal was in a previous environment, while the base reflects the most recent growth just before sampling. This method provides a timeline of dietary or habitat changes, which helps estimate time in captivity and detect possible transfers among holding sites.

The investigations leverage isoscapes developed specifically for Brazil, such as $\delta^{2}H$ maps for birds, $\delta^{15}N$ baselines for soils, and $\delta^{13}C$ distributions for tropical vegetation, enabling more accurate geographic assignments. Cluster analyses and probabilistic spatial models further strengthen the evidence, revealing links among animals from different locations and supporting enforcement actions to dismantle trafficking networks.

The Brazilian Federal Police's commitment to stable isotope forensics represents a scalable model to fight wildlife crime, combining laboratory protocols with spatial ecology to trace animal provenance and captivity histories, reinforcing species conservation and judicial accountability.

Potential for identification of the source of an explosive material based on analyses of the stable isotope ratios of residues

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with John D. Howa

We present field-based evidence applying stable isotope analyses to describe the isotope ratios of residues from detonations involving SEMTEX, PETN, TNT, and RDX, separately or in combination. An understanding of (a) the stable isotope ratios of residues recovered following a bombing and (b) clarifying the fractionation processes during that explosion could help link recovered residues to specific explosive manufacturers. Experimental testing was conducted in an open field at a U.S. government facility. The experiments involved controlled detonations of known explosives within individual 55-gallon barrel drums. Subsequently, residues from the walls of the drums were swabbed, explosive components isolated and identified, and stable isotope analyses conducted using IRMS instrumentation. In a second set of experiments, we analyzed explosive residues collected from soils, following detonations of bombs that had been dropped from aircraft. Both were analytical efforts to determine potential relationships between the stable isotope composition of explosive residues and that of the explosive material. The results of both sets of observations provide evidence of an analytical approach that could be applied to post-bomb analyses following any of a number of military- and IED-related events.

Using isotopic analysis to determine the origins of synthetic potassium chlorates used in explosives

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Potassium chlorate ($KClO_3$) has emerged as a cost-effective method to generate Improvised Explosive Devices. Developing tools capable of determining the origin of these illegally produced chlorates is critical to limit their trafficking. Due to the fact that isotopes vary predictably across the landscape with biogeochemical processes, they transmit geographic fingerprints to material generated at a given location. My research examines the potential of applying isotope geolocation to trace the provenance of chlorates. $KClO_3$ is most commonly synthesized by the electrolysis of water and potassium chloride (KCl).

The main objective of this study is to determine how chlorine and oxygen isotopes from the water and KCl precursors are transmitted to synthetic chlorates during electrolysis in well-controlled laboratory conditions. I synthesized $KClO_3$ by electrolysis using water and chlorine with distinct initial isotope compositions (-10.7‰, 0.2‰, 27.8‰, and 40.9‰ for $\delta^{18}O$, respectively, and 0‰, -4.61‰ and -20.83‰ for $\delta^{37}Cl$, respectively). As a prerequisite, ideal electrolysis conditions were determined in order to maximize the production and purity of ClO_3 in an electrolytic cell containing 25mL of electrolyte. We found that an electrolyte concentration of 0.2g/mL along with running the electrolysis at 4.0V for 9.5 hours consistently generated >95% chlorates with some KCl and KClO impurities, which could be entirely purified by adding a solubilization/recrystallization step.

Using this experimental design, a series of electrolysis experiments were run with the isotopically distinct precursors to test the transmission of O and Cl isotopes to chlorates. Both precursors and products were analyzed by isotope ratio mass spectrometry for $\delta^{18}O$ and $\delta^{37}Cl$. We found that $\delta^{18}O$ of the chlorates were linearly correlated to the $\delta^{18}O$ of the precursor water, with an equation of $\delta^{18}O_{chlorate} = 0.98\delta^{18}O_{water} - 27.41$ (α=0.97259, r^2=0.99, p-value=$2.53*10^{-29}$), supporting the direct transmission of water isotopes into the chlorates and the use of $\delta^{18}O$ values for chlorate attribution. Similarly, $\delta^{37}Cl$ is directly propagated from the KCl product to the chlorates.

While those results are promising, sample preparation, cost and time remain a major limitation to broadly apply this approach with forensic practitioners. We are testing the possible application of Oribtrap Exploris Isotope Solutions to streamline the combined analysis of O and Cl isotopes on chlorates to develop a more rapid and cost-effective solution for forensic investigators.

Isotopic characterisation of the homemade explosive (HME) R-salt

James F Carter

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with Yingua Wang & Tony Peter

R-salt (1,3,5-trinitroso-1,3,5-triazinane) is the nitroso form of the military high explosive RDX (1,3,5-trinitro-1,3,5-triazinane). Both R-salt and RDX are secondary high explosives with TNT equivalence of about 130% and 150% respectively. R-salt is not, however, used as a commercial or military explosive due to its limited chemical and thermal stability but has been associated with Middle Eastern terrorist attacks and the attempted 2025 New Year's Day attack in New Orleans.

Both R-salt and RDX are manufactured from hexamine, but R-salt can be prepared without access to concentrated nitric acid which is increasingly controlled as an explosive precursor. R-salt also has advantages over other HMEs, such as TATP (triacetone triperoxide) and HMTD (hexamethylene triperoxide diamine), that it is relatively insensitive to shock, friction and electrostatic discharge.

Our previous study investigated the stable isotopic compositions of hexamine, available in the form of commercial solid fuel tablets and used some of these samples to prepare HMTD. This earlier study identified a consistent change in both δ^{2}H and δ^{13}C compositions progressing from hexamine to HMTD with the potential to establish a link between the precursor and product.

For the present study, we purchased a suite of hexamine fuel tablets that were still commercially available following recent EU and UK restrictions on sale. We also obtained numerous samples of sodium nitrite which is another ingredient required to manufacture R-salt.

Samples of hexamine and sodium nitrite were analysed to determine their stable isotopic compositions, and selected samples were then used to synthesise R-salt. The samples of R-salt were, in turn, analysed to determine their isotopic compositions and to ascertain the relationship to the starting materials.

IRMS analysis of polymeric material for comparison casework

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Australian Federal Police/Griffith University

with Kylie Jones, Sarah Cresswell, Jim Carter, & Carney Matheson

Forensic polymer analysis performed at the Australian Federal Police (AFP) includes the use of IRMS analysis as part of our standard examination protocol. This compliments other instrumental techniques such as Fourier Transform Infrared Spectroscopy, which can elucidate differences between samples based on structure or Raman spectrometry which is useful when dyes or fillers are observed in the item being analysed. The combination of these three techniques has been observed to provide a strong discrimination potential between samples from different sources.

This presentation will discuss the general features of the isotope ratios of the population of different polymers and consumer products that have been measured, to support the interpretation when analysing casework samples for comparisons. Most of the analysis at the AFP has been performed on polyethylene products cling films, various bags including resealable, freezer bags and garbage bags. This has been supplemented by smaller populations of polypropylene ropes, polyvinyl chloride tapes, and 3D printer filaments from polylactic acid and other available polymers.

Specific questions that have been answered in the research have focused on material homogeneity, variability and changes that may be observed as part of the use and analysis of the products. An approach to reporting and several short case examples will be presented, demonstrating the utility and potential of IRMS for polymer evidence in a traditional forensic context.

Establishing baseline stable isotope data for humanitarian forensic tracing in Tanzania

Maximilian Jan Spies

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Migrants fleeing political or socio-economic instability are a recurring global phenomenon, both historically and in the present day. In East Africa, people from the Horn of Africa undergo perilous journeys through Tanzania en route to southern Africa and beyond. Sadly, many die, remaining unidentified, as bodies may be buried in unmarked graves or left in remote areas, leading to disarticulation or commingled remains that are hard to associate with individual identities. This humanitarian and forensic issue is severely limited by insufficient forensic infrastructure, including a lack of advanced DNA tools, forensic specialists, and isotopic reference data. Stable isotope analysis is an emerging forensic tracing tool, as isotope ratios incorporated into consumer tissues derive from food and water. These can therefore provide environmental, dietary, and cultural information that varies with population and geographic region. In this study, we investigated the variation in stable isotope ratios (δ^{13}C, δ^{15}N, and δ^{34}S) in human hair and nails donated by 106 living individuals residing along common smuggling routes in Tanzania to characterise local isotopic baselines. Values reflect diets that included a mixture of C3 (e.g. cassava, rice, bananas) and C4 crops (maize, sorghum, and millet). In wetter areas, mean δ^{15}N values were less than 10, and δ^{34}S was low. In drier areas, values were higher. As a group, the Tanzanian isotopic values differ from those in southern Ethiopia and from some communities in neighbouring Kenya. This study contributes to building regional isotopic reference databases. It demonstrates the potential of stable isotope analysis in this region as a complementary tool for tracing human movement, assisting with forensic identification, and understanding migration dynamics.

Disentangling dietary habit, sex differences, and tissue signature in human hair and nails via $\delta^{13}C$ analysis of bulk and individual amino acids

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Indian Institute of Science Education and Research Kolkata, India

with Prasanta Sanyal

The applications of stable isotopes in forensic science have seen significant advances in recent years. Bulk stable isotope analysis of C/N/H/O/S has proven to be an effective tool for identifying dietary habits, geographic origin, migration patterns and diet-related disorders. Stable isotope profiles have also been instrumental in forensic investigations. The carbon isotope ratio ($\delta^{13}C$) of proteinaceous tissues such as hair, nails, bone and teeth represents the averaged isotopic composition of the amino acids (AAs) that constitute them. Based on the human body's ability for de novo synthesis, AAs are classified as essential (E; those that must be obtained from diet) or non-essential (NE; those that can be synthesised in vivo). The EAAs are directly incorporated into tissues without significant alteration, and thus their isotopic signatures reflect dietary intake. In contrast, the isotopic composition of NEAAs is influenced not only by the quality of protein consumed, but also the metabolic pathways through which they are formed. Because of this, NEAAs can carry information about phenotypic traits such as sex, age, BMI, which would offer additional forensic insight. Further, multiple studies have reported that bulk $\delta^{13}C$ values of hair tissue are more positive compared to nail tissue. This variation is generally attributed to differences in the amino acid composition between the two tissues, although there is no clear insight into the specific AAs responsible for the same.

In this study, we aim to utilise the $\delta^{13}C$ values of bulk and 11 individual AAs to determine the variables that influence dietary habit, sex, and sample type. Scalp hair and fingernail samples were collected from 35 healthy participants (20 females, 15 males) aged 20-30 years with a mean BMI of 23 ± 3.7. The $\delta^{13}C$ values of hair and nail samples were analysed via EA-IRMS for bulk and GC-IRMS for individual AAs.

We observed that the branched chain AAs (Leu, Val, Ile) and aromatic AAs (Phe, Tyr) accounted for the greatest proportion of variance across all samples. Among EAAs, Leu, Val, Phe, and Tyr were most influenced by diet and a random forest model achieved up to 80% accuracy in classifying participants as lacto-vegetarian or omnivore. Sex-related differences were most visible in Tyr and Phe, and we achieved 66% accuracy in sex classification. Hair and nail tissues exhibited distinct isotope signatures for $\delta^{13}C$ values of bulk, Gly and Tyr, with lower $\delta^{13}C$ values in nail compared to hair. In contrast, Val and Leu $\delta^{13}C$ values were identical between hair and nails (slope = 1), suggesting uniform incorporation, whereas the $\delta^{13}C$ values of Lys, Ala and Asx implied tissue-specific inheritance (slope = 0). These findings represent that isotope analysis of AAs is a valuable next step in forensic investigations, both for human identification and for understanding metabolic pathways of dietary amino acid assimilation.

Assessing C and H isotopic fractionation of aliphatic hydrocarbons during microbial degradation: Microcosm studies and environmental implications

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with Russell D. Frew, Alan R. Hayman, & Robert Van Hale

Microbial degradation of hydrocarbons has been widely studied for remediation purposes. However, there are still unquantifiable factors that are not properly understood because of the limitation with the conventional techniques used. Stable isotope analysis has been applied as an alternative approach to the conventional method. The unique stable isotope fingerprints of hydrocarbon compounds make them viable to be applied in source-apportionment of an oil spill due to its stability undergoing various processes. On the contrary, changes in the stable isotope compositions of the hydrocarbon discovered to occur during physical and biological weathering makes them useful in the application of this method in monitoring the occurrence of in situ biodegradation and estimating the rates and extent of degradation. Hence, we carried out microcosm studies to monitor and evaluate the microbial degradation of the aliphatic hydrocarbons following an oil spill using compound-specific isotope analysis. The effects of microbial degradation on the isotopic compositions of the hydrocarbons will be tested using Rayleigh model in order to assess the state and behaviour of the system in operation. Based on the data in this study, the findings indicated that carbon isotopic fractionation patterns were different from that of hydrogen isotopes. Small carbon isotope enrichment in the substrates of n-alkanes could only be detected in $n\mathrm{C}_{12}$ and $n\mathrm{C}_{13}$ after slight to moderate biodegradation. On the other hand, $^{2}\mathrm{H}$ enrichment in $n\mathrm{C}_{12}$ to $n\mathrm{C}_{19}$ occurred very early during the biodegradation process with high values of enrichment. The trends observed in the $\delta^{2}\mathrm{H}$ shifts in the *n*-alkanes caused by microbial degradation, generally followed the Rayleigh model. In summary, CSIA has been successful in providing fundamental data on the effects of biodegradation by efficiently demonstrating the magnitude and direction of isotopic shift occurring in aliphatic hydrocarbons.

Implementation of the stable isotope analysis laboratory at the National Institute of Criminalistics of the Brazilian Federal Police: First investigations aided using the technology

J.M. Freitas

National Institute of Criminalistics of Brazilian Federal Police

with F.C. Aquino, E.D. Botelho, & M.L. Vieira

The Stable Isotope Analysis Laboratory of the Brazilian Federal Police (PF) at the National Institute of Criminalistics (INC), began operations in 2021 and has been establishing itself as an important source for evidence characterization. The laboratory is part of the Laboratory Forensics Service (SEPLAB), which has been accredited under ISO 17025 since 2014.

The initial steps at the new laboratory were the validation of the installed instruments. The first validated methodology was the analysis of carbon and nitrogen using CSIA-IRMS and oxygen and hydrogen by EA-IRMS in cocaine samples. To date 560 samples have been analyzed, primarily as a new layer at the PF profiling cocaine program for the construction of databases aimed at the geographical attribution of samples, but also in 7 forensic cases, where the goal was to evaluate possible correlations between different seizures.

The laboratory also participated in the analysis of seizures from police operations investigating honey adulteration by the addition of cane sugar and butter adulteration by the addition of soybean and palm vegetable oils. Carbon isotopes were analyzed in 175 samples, with approximately 70% being adulterated in the case of honey and 90% in the case of butter. Analyses of counterfeit alcoholic beverages were also carried out by CSIA-IRMS, detecting the addition of C_4 plant-derived alcohol to beverages such as whiskey and vodka.

Currently, the laboratory is dedicated to establishing methodologies for the analysis of human biological tissues. Brazil has a large backlog of unidentified human remains, and isotopic analysis can help in many of these cases. Methodologies for the analysis of C, N, S, O, and H in keratinized tissues (nails and hair) and C and N in collagen obtained from bones and dentin have already been implemented.

Isotopic fingerprinting of illegal timber: A provenance tool for the amazon basin

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with Ana Claudia Batista, Maria Gabriela Araujo, Isabela Souza-Silva, & Gabriela Nardoto

Illegal logging remains one of the most critical threats to the conservation of tropical forests, particularly in the Amazon Basin. To address this challenge, we developed an isotopic provenance system capable of determining the geographic origin of timber and detecting illegally harvested wood. We sampled 650 trees across 58 locations throughout the Brazilian Amazon and measured four isotopic markers: $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, and $^{87}Sr/^{86}Sr$. These data were used to construct high-resolution isoscapes for each isotope. Using the R package assignR, which applies a Bayesian framework for geographic assignment, we created a probabilistic system capable of identifying the most likely origin of unknown timber samples. This methodology enables robust forensic identification by comparing wood samples of uncertain origin to our spatial isotope reference database. Designed to support Brazilian law-enforcement agencies in combating illegal logging, the system also provides a verification tool for timber exporters, importers, and certification bodies seeking to ensure supply chain transparency. Our findings demonstrate that combining multiple stable and radiogenic isotope systems greatly improves the accuracy and geographic resolution of timber provenance in a region where regulatory enforcement is limited and illegal extraction widespread.

(WITHDRAWN) Forensic determination of tropical timber provenance using strontium isotopes and multi-element signatures: Development and validation of a geochemical fingerprinting protocol

Camilla Kafino

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with Artur Moraes de Amorim, Tarcisio Almeida, Roberto Ventura Santos, & Christophe Pecheyran

Illegal logging and timber fraud undermine conservation efforts and forest law enforcement. To address this, we developed a protocol for geographic origin attribution of tropical timber based on strontium isotope ratios ($^{87}Sr/^{86}Sr$), complemented by multi-elemental analysis. Wood, soil (bulk and labile fractions), and rock samples were collected across two distinct Brazilian biomes—the Atlantic Forest and the Amazon.

The results demonstrate that $^{87}Sr/^{86}Sr$ values vary significantly between geologic formations ($p = 0.05$), establishing $^{87}Sr/^{86}Sr$ as a reliable geolocation marker. The labile fraction of the soil was confirmed as the primary bioavailable Sr source, with transfer factors close to unity (1.0023 ± 0.0031), validating the soil–wood isotopic continuity.

Multi-elemental signatures, including Sr/Ba ratios and marine aerosol influence indices, further enhanced spatial resolution, particularly within geologically homogeneous units. The integration of isotopic and elemental data improved discrimination power, enabling origin attribution even among closely situated locations.

Based on these findings, we propose the Wood Provenance Attribution Protocol (AOM), a forensic tool capable of verifying the declared origin of timber through intrinsic geochemical properties, immune to document-based fraud. This method can be integrated into certification schemes, enforcement protocols, and legal investigations. Its validation in tropical contexts—including endangered species like pau-brasil—demonstrates its transformative potential for combating illegal logging and supporting global timber traceability systems.

World Forest ID: Large scale stable isotope ratio modeling for product verification

Victor Deklerck
World Forest ID

with Jakub Truszkowski, Charlotte Smith, & Jade Saunders

Although stable isotope ratio analysis has proven effective in determining the origin of natural products, its broader application is constrained by two main factors: (1) the lack of comprehensive reference datasets and (2) challenges in model development. World Forest ID aims to overcome these limitations by creating large-scale spatial models, based on stable isotope ratio data and grounded in extensive georeferenced reference databases. These databases include stable isotope ratio data for timber, soy, cacao, and coffee, among others.

Samples are collected according to standardized protocols and transported to partner collection institutions. Subsamples are then sent to analytical laboratories to measure their stable isotope ratios (δ^{2}H, δ^{13}C, δ^{15}N, δ^{18}O, δ^{34}S). The resulting data is fed into Gaussian Process regression models, enhanced with Bayesian inference, enabling both the determination of harvest origin (“Where does this come from?”) and the verification of claimed origins (“Is this claim plausible?”). These assessments can be made at multiple spatial resolutions, from the country level down to specific GPS coordinates. Laboratories can test samples against these models on the World Forest ID spatial platform, which will automatically generate a report.

A practical example of this pipeline is the tracing of conflict timber in the wake of the Ukraine invasion. Timber shipments suspected of originating from Russia or Belarus are routinely tested against these models to assess their harvest location claim.

Applications of stable isotope ratio analysis and site-specific natural isotope fractionation-nuclear magnetic resonance in discriminating between synthetic and natural analogs

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with Silvia Pianezze

Consumers today are increasingly seeking products containing molecules of natural origin, as these are often perceived as healthier compared to their synthetic or semi-synthetic counterparts. However, the higher costs associated with the production of plant-based raw materials, as well as the extraction and purification of these natural substances, create opportunities for counterfeiting. This often involves the addition of cheaper, chemically indistinguishable synthetic forms. Techniques such as Stable Isotope Ratio Mass Spectrometry (SIRA) and Site-Specific Natural Isotope Fractionation by Nuclear Magnetic Resonance (SNIF-NMR) are valuable tools for distinguishing molecules of natural, biosynthetic, or synthetic origin across various categories, including flavorings, essential oils, foodstuffs, dietary supplements, pharmaceuticals, and steroids. While both methods are expensive and require specialized equipment, SNIF-NMR is less accessible and more complex than SIRA. However, it provides more detailed, site-specific molecular information. Despite the high precision and sensitivity of SNIF-NMR, SIRA offers broader scientific applications but is challenged by complex data interpretation and the limited availability of reference materials. Among isotopic parameters, δ^{2}H measured by IRMS or (D/H)n determined via SNIF-NMR has demonstrated the greatest ability to discriminate, generally showing lower values in natural molecules and more positive ones in their synthetic counterparts. Although δ^{13}C is the most extensively studied parameter, it does not always provide significant discrimination between natural and synthetic fossil-derived products. Nevertheless, it is particularly effective for differentiating natural molecules extracted from specific plants and their biosynthetic analogs, which are synthesized from C4 substrates such as sugarcane and corn (e.g., red yeast rice or L-theanine). In specific cases, δ^{15}N (e.g., for caffeine) and δ^{18}O (e.g., for *Serenoa repens* extract) have shown excellent potential for characterization.

Stable isotope analysis of *Paubrasilia echinata* violin bows: Insights into the illegal exploration of an endangered tree species.

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with Jose Haroldo de Oliveira, Jorge Marcelo de Freitas, Andrea Aparecida Sargi, & Vladimir Eliodoro Costa

On the 30th of November 2021, two forensic experts of the Federal Police of Brazil were requested to support the investigation team on a police operation dedicated to the repression of illegal logging of the CITES regulated tree species *Paubrasilia echinata*. On the occasion, four search and seizure warrants were served, and a total of 2,869 violin bow sticks were apprehended. Brazilwood or Pernambuco wood (*Paubrasilia echinata*) is an endangered tree species endemic to the Brazilian Atlantic Forest that can reach 15 metres in height and has a characteristic bright red colour to its sapwood. The species used to be abundant along the coast of Brazil, but due to unsustainable exploration during the colonization period for the red dye that was produced from it, brazilwood became extremely rare, now being cited in the official list of endangered flora of Brazil. Nowadays, it can be used in the confection of musical instruments, including friction string instrument bows that can be sold for up to 5,000.00 USD. On the occasion, the accusation hypothesis was that the apprehended violin bow sticks were produced using timber illegally harvested from the "PARNA do Pau Brasil," a national park that preserves an important remnant of the Atlantic Forest biome. 107 violin bow sticks were collected by the forensics team and were analysed for $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{2}H$ with a DeltaV Advantage IRMS, with a specific preparation procedure and C and N acquisition method that was developed. The results were compared to those of reference samples from a legal brazilwood crop as well as protected national park trees using Hierarchical Cluster Analysis and Quadratic Discriminant Analysis to evaluate the compatibility of questioned and reference samples. 23 of the violin bow stick samples were considered more compatible with the protected tree samples from the national park.

Stable isotope fingerprinting of tomato processing: A forensic approach to food authenticity and traceability

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with Ana Maria Simionescu, Diana Costinel, & Roxana Elena Ionete

The forensic potential of stable isotope ratio analysis (SIRA) in verifying the authenticity and traceability of food products is increasingly recognized. This study investigates the isotopic behavior of tomato matrices subjected to thermal processing and applies the findings to evaluate commercial tomato-based products for potential adulteration. Experimental trials were conducted on six tomato varieties processed into concentrated passata (Brix ~36). Isotope ratio mass spectrometry (IRMS) was used to measure $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, and $\delta^{2}H$ in both whole samples and isolated fractions (pectin, pulp, sugars, organic acids, and ethanol derived via fermentation). Despite substantial water removal, $\delta^{13}C$ and $\delta^{15}N$ values exhibited high stability across processing steps. Intra-fraction differences in $\delta^{13}C$ were generally under 1‰, with $\delta^{15}N$ showing minimal variability across bulk and nitrogen-containing subfractions. $\delta^{18}O$ and $\delta^{2}H$ values increased progressively, reflecting evaporative enrichment under vacuum processing; these shifts were strongly correlated with concentration time ($R^2 > 0.98$). To assess forensic relevance, a comparative analysis was extended to a panel of commercial samples. Multivariate techniques, including principal component analysis (PCA) and hierarchical clustering (HCA), revealed that authentic samples formed tight clusters, while several commercial products displayed significant isotopic divergence. In particular, anomalies in $\delta^{13}C$ ethanol and $\delta^{15}N$ bulk suggested possible formulation differences or undeclared ingredient origins. Correlations among isotope systems (e.g., $\delta^{13}C$ ethanol–$\delta^{13}C$ pulp: R = 0.68; $\delta^{15}N$ pectin–$\delta^{15}N$ pulp: R = 0.81) supported the use of inter-fractional relationships as diagnostic markers. The combination of matrix-specific isotope profiles, evaporation-sensitive parameters, and multivariate data analysis offers a robust framework for the forensic assessment of tomato-derived products. These findings demonstrate the capacity of SIRA not only to monitor processing effects but also to flag compositional inconsistencies in the commercial supply chain.

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Evaluation of measurement uncertainty during inter-laboratory characterization of isotope delta reference materials and lessons for routine analyses

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with Simon Cowen

The quality of analytical measurements depends on traceability and uncertainty amongst other attributes. For isotope delta, reference materials (RMs) play a vital role for both – they are how traceability is assured while their assigned and measured uncertainties are both important components of uncertainty budgets for routine measurements.

Characterization of RMs for isotope delta has often been performed by inter-laboratory exercise bringing the advantage of greater confidence in the assigned values but also resulting in more complex traceability chains and more challenging evaluation of uncertainty. Indeed, some recently released RMs have been criticized for having assigned uncertainties that are too small, potentially breaking traceability chains as each calibration step should result in an increase in measurement uncertainty (Gröning 2023).

We have reviewed calibration methods employed during interlaboratory characterization of new RMs where measured instrumental data were available. We have then propagated measurement uncertainties both within and between laboratories during centralized calibration of measured isotope delta values. This has included single-point calibration of DI-IRMS results as well as two-point and multiple-point calibration of CF-IRMS results. This approach allowed complete separation of random from systematic sources of uncertainty through each calculation stage. It also allowed correlations among laboratories arising from the use of the same RMs for calibration to be accounted for.

Similar methods can be employed for uncertainty estimation for calibration within single laboratories during routine analyses, to combine independent measurements during characterization of in-house RMs or to process results from method validation experiments. The derived uncertainty models also provide guidance on experimental design regarding selection of RMs for calibration.

Reference:
Gröning M., Accred. Qual. Assur., 2023, 28, 101-114

Guidance for stable isotope databases

Ethan Strak

Food Forensics

with Phil Dunn, Chris Brodie, Gabe Bowen, Glen Jackson, Helen Salouros, Jason West, Jim Carter, Simon Kelly, Kylie Jones, Lesley Chesson, Joe Meikle, & Nives Ogrinc

Many forensic applications of isotopic analyses depend on the establishment, population and querying of databases of measurement results. While the FIRMS Good Practice Guide for IRMS provides guidance on the processes involved in making measurements, there is no similar guidance for databases of isotopic analyses.

The FIRMS Network is currently drafting guidance consisting of three broad areas concerning isotope ratio databases: (1) data quality and assessment of data quality as this is the foundation of a database that can be relied on in a forensic context; (2) creation and population of databases of isotope ratio data focussing on ensuring data consistency; and (3) “use” of a database or interpretation of measurement results in comparison to those in a database.

This short presentation will briefly introduce the draft guidance to stimulate feedback and suggestions on topics and issues for FIRMS to include and address.