

Network Developing Forensic Applications of Stable Isotope Ratio Mass Spectrometry Conference 2002.

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Introduction

The Forensic Isotope Ratio Mass Spectrometry (FIRMS) Network was formed in 2002 by Prof. Max Coleman of Reading University and Sean Doyle of the Forensic Explosives Laboratory, Dstl. The network is funded for three years by the Engineering and Physical Sciences Research Council (EPSRC) following a call for forensic network proposals under the UK Government Foresight programme.

As part of the network development a conference was held at the Thistle Hotel, Brands Hatch, Kent on the 16th and 17th September 2002. The conference, additionally sponsored by Dstl, Micromass, ThermoFinnigan, Pelican Scientific and PDZ Europa Ltd, was attended by over 50 delegates from forensic establishments, police forces and academia both within the UK and internationally. The conference aimed to bring together IRMS researchers, end users and instrument manufacturers. The objectives of the conference were to:

- Exchange information between the researchers and end users.
- Expand the network.
- Understand the statement of requirement.
- Formulate a strategy for development.
- Determine interest in a workshop to define research priorities.

The conference was a mix of presentations and discussion sessions. A summary of each presentation and the discussions are given below.

Stable Isotope Characterisation – How it works, why it works and how good it is.
Prof. Max Coleman, Postgraduate Research Institute for Sedimentology, University of Reading, Whiteknights, Reading, RG6 6AB, UK.

The first presentation aimed to welcome delegates to the conference and to introduce the topics that would be dealt with in subsequent presentations.

Stable isotope determinations are unlike other chemical measurements in that the primary methodology produces very precise results but accuracy is not relevant as all values are presented relative to international reference materials. Traditional stable isotope analysis for most light elements involves a chemical preparation stage that produces a gaseous species from the material to be analysed, which is introduced into a dual inlet mass spectrometer alternately with gas produced from a reference material. While dual inlet is the most precise method for stable isotope ratio measurement continuous flow mass spectrometry lends itself to on-line sample preparation, smaller sample size, faster analysis and the possibility of interfacing with other separation techniques like gas chromatography, but at the cost of poorer precision. Table 1 shows the abundances of the most commonly measured isotopes.

Major isotope	Largest trace isotope	ppm relative to largest isotope
¹ H	^D H	158
¹² C	¹³ C	11,000
¹⁴ N	¹⁵ N	3,700
¹⁶ O	¹⁸ O	2,000
³² S	³⁴ S	42,000
³⁵ Cl	³⁷ Cl	244,700

Table 1. Abundance of commonly measured isotopes.
 Stable isotope measurements are expressed in delta values:

$$\delta^{13}\text{C} (\text{‰}) = \frac{(R_{\text{samp}}^{13} - R_{\text{std}}^{13})}{R_{\text{std}}^{13}} \times 10^3$$

where $R^{13} = {}^{13}\text{C} / {}^{12}\text{C}$

Positive delta values indicate that there is a greater percentage of the heavier isotope present relative to the standard. The most commonly measured elements are H, Li, B, C, N, O, Si and S. Characterisation of materials based on their stable isotope ratios relies on a natural range of values and its extension resulting from manufacturing processes which may give rise to isotopic fractionation. However, isotopic homogeneity or heterogeneity are also valuable characteristics. An essential consideration is sample integrity and its ability to retain its isotopic compositions. Some materials are susceptible to microbial attack, which may profoundly affect isotope compositions of the partially destroyed residual material. The use of one isotope may give useful characterisation but the likelihood of uniqueness increases when more than one isotope is measured. The presentation summarised that stable isotope analysis, like any other technique, is only as good as the care taken in using it.

Isotopes as a Tool to Evaluate Abiotic and Biochemical Reactions. Complicating Issues for Forensic Applications of IRMS: Examples Using Environmental Forensics.

Prof. Robert Kalin, Queens University, Belfast, Northern Ireland.

Isotope ratio mass spectrometry has been applied to a number of forensic science disciplines. However, before applying IRMS to forensic science a greater understanding of the processes that govern the stable isotope ratios must be sought.

- What is the initial isotope content?
- Does it change during abiotic or biological reactions?
- If it does change, can this change be used?
- Does any change relate to a process that can be used to understand a biological mechanism?
- Can we use these changes to confirm or refute a hypothesised reaction?

The presentation gave examples of the application of IRMS to environmental forensic science. The first example given was the problem of environmental contamination with methyl tert-butyl ether (MTBE) found in gasoline. Analysis of approximately 80 gasoline samples collected globally showed only a slight variation in the ^{13}C values. The question needs to be asked though of whether these values are conserved in nature. Experiments performed studying the conservation of the stable isotope ratios when the MTBE undergoes oxidation with potassium permanganate (an example of an abiotic reaction) showed that there was a 10 permil change in the ^{13}C ratio. Studies of field isolated consortia showed that there was a 0 permil change indicating no evidence that the MTBE was being broken down in the environment.

Similar studies have been carried out with trichloroethene (TCE). Studies of the effects of abiotic, anaerobic and aerobic reactions of TCE on the stable isotope ratios were performed, both field based and lab based. The studies showed that abiotic reactions were different to microbial reactions and that anaerobic reactions were different to aerobic reactions. Similar studies were performed on the transhalogenation of bromomethane and chloromethane where biological systems were compared with abiotic systems.

The presentation summarised that abiotic and biologically mediated reactions can change the isotopic composition of target compounds. Care must be taken to determine if a degradation reaction has taken place or not. When degradation has taken place the changes in isotope compositions should be evaluated. Isotope changes can be used as both positive and null hypothesis results. Future research will include studies to gain a validated understanding of the fate of compounds in the natural environment.

Stable Isotope Ratio Profiling. The Challenge and the Vision.

Sean Doyle, Forensic Explosives Laboratory, Dstl, Fort Halstead, Sevenoaks, Kent TN14 7BP, United Kingdom.

In 1999 the Forensic Explosives Laboratory (FEL) commenced a three year Home Office funded project to investigate the use of stable isotope ratios in the forensic analysis of explosives. Today, in 2002, the FEL has a suite of methods for the analysis of elements of explosives significance and in 2003 the FEL plan to take delivery of a CF/DI-IRMS, funded by the Home Office.

The first year of the three-year project was carried out by PDZ Europa using EA CF-IRMS for carbon and nitrogen stable isotope analysis of commercial and improvised high and low explosives. The second year continued with the development of methods by Iso-Analytical for carbon, nitrogen, oxygen and sulphur stable isotope ratios of specific explosives and the development of a method by the University of Reading for the analysis of chlorine stable isotopes in chlorate and perchlorate based explosives. The final year of the project investigated batch variation, the further development of a method for the analysis of oxygen and validation of the chlorine method.

The validation of any IRMS method developed for forensic use is of primary importance. Validation of the method is required, as it will provide analytical results that will be used as evidence in a court of law. In high profile terrorist or organised crime trials evidence tendered in the jurisdictions of the developed world is likely to be contested by the most skilled lawyers. International collaboration on the development and validation of such methods is therefore essential from the outset. The validation process should include the following:

- Proof of concept.
- Method development.
- Publication in a peer reviewed journal.
- UKAS accreditation.

The method also requires validating forensically to ensure that it is providing relevant evidence of probative value. Thought needs to be given as to how this degree of validation might be achieved, aided through knowledge of past miscarriages of justice. Development and validation of forensic methods must involve all the stakeholders including researchers, developers, policy makers, instrument manufacturers, practitioners, investigators and lawyers. The use for this type of analysis extends beyond that which is to be used as evidence in a legal process to evidence which can be used for intelligence purposes, to be used proactively for the prevention or reduction of crime. This may require stable light isotope ratio databases, similar to current DNA and fingerprint databases, the population of which will be a large undertaking requiring a clear understanding of the end user requirements. There is a need to work towards internationally agreed standard methods for data acquisition. There is a strong need to burden share, to avoid duplication of effort, and this will require a global task allocation for data acquisition, database population and the provision of data sharing with secure access. To achieve this research and development needs will require precise identification with input from the end users. The instrument manufacturers will need to reflect on the need for high throughput of samples and researchers need to consider trace requirements and improved sensitivity.

In the discussion it was recognised that there are other groups within the forensic field with similar problems of building databases. Discussion with these groups may prove beneficial. The similarity of this network with the European NITE-CRIME network was also recognised and it was envisaged that they would complement each other. When comparing results between laboratories the importance of the use of traceable standards that have been measured relative to international standards was discussed. This could prove to be a problem if laboratories use their own standards only traceable to themselves and this will raise much criticism of the technique.

End User Requirements - Discussion Session.

Chair: Dr Mark Stroud, Dstl, Fort Halstead, Sevenoaks, Kent TN14 7BP, UK.

Data produced by isotope ratio mass spectrometry when used in the legal forum must be of a clear, agreed evidential standard. The results must be relatively rapid to obtain, must not be overly expensive and must be understandable i.e. they must be able to be explained to a judge and jury.

One of the main problems envisaged is the use of traceable standards for analysis, standards that ideally should be matrix matched to the sample. The list of required traceable standards will be long and is a priority for future discussions through the use of defined working groups. It was thought that the use of identical methods are not essential as long as each laboratories method gives identical results. Advice and guidance should be sought from accrediting bodies such as UKAS. This would require the use of round robin collaborative exercises.

Another area for discussion is the use and development of relevant databases. While the databases cannot be established before the methods are developed and validated thought will need to be given as to the type of databases required, how they will be shared between laboratories and who will fund their development and maintenance. Fortunately there are many forensic databases already in operation will serve as models for future isotope ratio databases.

Application of EA-C-CF-IRMS in the Analysis of Explosives.

Charles Belanger, Iso-Analytical Ltd, Unit 2 Zan Industrial Estate, Sandbach, Cheshire, CW11 4QD, UK.

The presentation began by giving a general overview of the operation of the laboratory at Iso-Analytical before detailing the initial studies and method development carried out for the Forensic Explosives Laboratory in the analysis of explosives.

During 2000-2002 Iso-Analytical undertook the analysis of a number of explosives samples for the Forensic Explosives Laboratory, initially to establish whether there was a range of carbon and nitrogen stable isotope ratio values.

^{15}N and ^{13}C were determined by isotope ratio measurement of N_2 and CO_2 gases respectively. Samples weighed into tin foil capsules undergo Dumas combustion at a temperature of 1700°C in the presence of O_2 . Flash combustion of the tin capsule increases the temperature in the furnace. The gases undergo primary oxidation at 1000°C on a CrO_3 catalyst. Hydrocarbons are combusted on a CuO catalyst and Ag removes any sulphur or halides. The resulting gases of CO_2 , N_2 , NO_x , H_2O and O_2 undergo reduction on high purity reduced Cu(II) at 600°C which removes excess O_2 and reduces the NO_x to N_2 . The remaining gases, N_2 , CO_2 and H_2O , pass through chemical traps of Carbosorb to remove the CO_2 (if not required for ^{13}C analysis) and/or $\text{Mg(ClO}_4)_2$ to remove the H_2O . The N_2 (and CO_2) passes through a GC column before entering the mass spectrometer.

The analysis of samples of ammonium nitrate, gunpowder, TNT and plastic explosive confirmed there was indeed a range of values for carbon and nitrogen isotope ratios and that the technique had the potential for the forensic analysis of explosives. Further studies were undertaken to additionally measure sulphur and oxygen isotope ratios.

Sulphur analysis of gunpowder samples proved to be problematic. It was found that the CO_2 produced from the high carbon content samples interfered with the SO_2 peak on the chromatograph. The SO_2 peak itself was prone to tailing on the GC and in the mass spectrum there was interference from ^{18}O . These factors were overcome by diverting the carbon dioxide peak to atmosphere, integrating the entire sulphur dioxide peak and correcting for the ^{18}O by analysing a range of accepted standards and generating a correction curve.

^{18}O was determined by isotope ratio measurement of CO (masses 28, 29 and 30). Analyses were performed in silver foil capsules with pyrolysis at 1080°C with glassy carbon chips. The resulting CO was resolved from nitrogen and hydrogen by a packed molecular sieve GC column. Problems were encountered with the conversion rates for RDX and nitroglycerine (only 65% and 79% respectively). The use of 50% nickelised carbon with pyrolysis at 1080°C with glassy carbon chips improved the conversion rates to 97% for RDX and 100% for nitroglycerine.

In discussion the question of combined uncertainty by using primary, secondary and tertiary standards was raised. It was thought that few people have probably calculated this uncertainty and that error propagation was often neglected in this field.

Panel Discussion - Day One Speakers

Panel members: Sean Doyle, Prof. Max Coleman, Prof. Robert Kalin and Dr Charles Belanger.

Questions were put to the panel regarding the use of IRMS in the field of forensic science. The panel envisaged that the technique may have been developed sufficiently within the next five years to be used evidentially in a court of law. However, there is much work that needs to be done prior to this and complications may arise along the way. Care must be taken when developing the method so as not to jeopardise the first evidence coming from this technique. Even for intelligence purposes the evidence needs to be robust and as such should be using validated techniques. At present the technique has the ability to produce data which will say whether two samples are consistent or not consistent with each other. With the use of databases samples can be searched against reference materials of known origin. The research required to develop the use of IRMS in the field of forensic science must be prioritised. While terrorism and drugs crimes are topical the technique also has many other potential applications i.e. glass, paint, paper and fibres.

Forensics and Terrorism: Useful Stable Isotope Approaches.

Prof. James Ehleringer, Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, Utah, USA.

Stable isotope ratio analysis is well established in fields such as environmental science, geology, biology and oceanography but has limited forensic applications to date. It has become more established in forensic fields such as food adulteration and the arts but recent advances in the field of IRMS have opened up other applications, one of those being terrorism.

Stable isotope ratio analysis can be used as an additional tool to corroborate and confirm many evidential leads in the investigation of terrorism. One potential use is in geo-location, that is the relationship between stable isotopes and location. An example of this is the recording of stable isotopes of water from different locations in human blood. Due to a process known as Rayleigh distribution water from different geographic locations has different stable isotope ratios. The turnover of water in the human body is approximately twelve days and therefore human blood records the isotope ratios of the local water sources and hence carries a history of what a person has been drinking and eating. The analysis of CO₂ in human breath can be linked to recent location. Isotope ratios of the environment are also recorded in bone and teeth.

Stable isotope ratios can be used to provide information about the origin of counterfeit money that may have been used to fund terrorist activities. By measurement of the hydrogen and oxygen stable isotope ratios in the paper the source of the cellulose can be determined.

Stable isotope ratio analysis can also be used in the investigation of drugs crime. It can be used to distinguish between different countries of manufacture or different manufacturers of pharmaceutical drugs and can be used to source the origins of drugs such as heroin. Differences in the growing regions of the drug plant will lead to differences in the nitrogen, carbon and oxygen stable isotope ratios.

Stable isotope ratio analysis has also been applied to explosives. Studies with PETN have shown that PETN produced from the same manufacturer falls within a tight cluster of stable isotope ratio values, distinguishable from other manufacturers. Therefore, information can be obtained as to whether a suspect sample is consistent with being produced by a certain manufacturer.

The threat from biological weapons is also very real. In the same way that the stable isotopes of water from different geo-locations is recorded in the human body this is also true for microorganisms.

In summary the keys to success in the forensic ecology of terrorism were given below:

- Identify end user products.
- Start off simple i.e. chose a few applications to begin with.
- Develop databases.
- Recognise globalisation and economics.
- Improved links between universities and forensic laboratories.

In discussion Prof. Ehleringer stated that the reflection of water stable isotopes ratios in breath gas and water was not effected by an individuals metabolism as the fractionation rules in the human body are known. However, these rules only apply when the body is resting and will change during extreme exercise. The point was raised that when paper samples are analysed for ^{18}O signatures it is important to keep the paper samples dry so as to avoid contribution by moisture.

Isotopic Characterisation of Ecstasy.

Dr James Carter, School of Chemistry, University of Bristol, Cantocks Close, Bristol, BS8 1TS, UK.

Ecstasy (3,4-methylenedioxyamphetamine) is typically prepared from a number of cheap and readily available natural products via conversion to 3,4-methylenedioxyphenylacetone and reductive amination to form an N-substituted amine. The study aimed to use the isotopic fingerprint of the drug to distinguish between different batches, to link bulk and trace evidence, to trace drugs to a common source of manufacture or supply and to determine the synthetic route.

In the first study five batches of ecstasy tablets, comprising ten tablets per batch, were analysed. The tablets were crushed and dissolved in an aqueous base and the active ingredients extracted using a liquid/liquid extraction prior to analysis by GC/MS, NMR and isotope ratio mass spectrometry (for hydrogen, carbon and nitrogen isotopes).

^{13}C analysis was carried out using a ThermoFinnigan Delta^{PLUS}XL IRMS with a GCC III interface, a Cu/Ni/Pt oxidation reactor at 950°C and Cu reduction reactor at 600°C. The instrument was calibrated to NIST sucrose (-10.47‰ vs VPDB). The ten tablets within each batch were found to be indistinguishable from each other. MDA extracted from one batch of tablets was found to be >2.5‰ heavier in ^{13}C than the four batches of MDMA, suggesting significant contribution from the N-methyl group to the signature of the MDMA. Much smaller variations in $\delta^{13}\text{C}$ were observed between the batches of MDMA.

^{15}N analysis was performed on a CarloErba 2500 EA ThermoFinnigan Delta^{PLUS}XL, using a Cr oxide and Ag/Co oxide at 1050°C and Cu reduction at 640°C. The instrument was calibrated to NIST ammonium sulphate (0.4‰ and 20.3‰ vs air). Each batch of tablets was found to have a unique $\delta^{15}\text{N}$ value.

^2H analysis was performed on a ThermoFinnigan Delta^{PLUS}XL GC-TC-IRMS instrument with thermal conversion at 1450°C. The instrument was calibrated to a suite of n-alkanes (C_{16} – C_{30} calibrated against VSMOW). Four of the five batches of tablets were isotopically indistinct while one batch of MDMA was approximately 22% depleted, possibly suggesting a different synthetic origin. ^2H SNIF-NMR (stable natural isotope fractionation-NMR) analysis was performed on the extracted MDMA using a Jeol Alpha 500MHz instrument, using 500-1000mg analyte in CHCl_3 with 30,000 scans in 22 hours. The data revealed that variations in the degrees of deuterium substitution occurred primarily at the methylene and terminal methyl groups, consistent with the known synthesis of MDMA.

The combined $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^2\text{H}$ data gave five distinct populations and provided a means to discriminate batches of illicit MDMA and MDA.

The second study aimed to predict the metamphetamine signatures from the known signatures of the precursors. There was a good match to the expected value for the $\delta^{13}\text{C}$ results. However, a large shift was obtained for the $\delta^{15}\text{N}$ values, the values of which were not reproducible with repeated syntheses.

In summary the studies concluded that IRMS provides a fingerprint of the drug. $\delta^{15}\text{N}$ data is a major discriminating factor with the $\delta^{13}\text{C}$ and $\delta^2\text{H}$ as minor discriminating factors. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data reflect the reductive amination process while the $\delta^2\text{H}$ reflects the origin and solvent history. IRMS and ^2H -NMR reveal the synthesis route. The analysis is applicable to only a few hundred nanograms of material.

Discussions centred on the experiments to study the predicted stable isotope ratio values of synthesised MDMA. As the synthesis is carried out with an excess of one reagent isotopic fractionation is expected to occur. The speed of the addition of reagents will also effect the fractionation. As there was no variation in the delta values of the substrates for the different syntheses the variation in end product delta values is due to the synthesis method and not due to differences in the substrates.

Stable Isotopic Characterisation of Drug Substances and Drug Products.

Dr John P. Jasper, Molecular Isotope Technologies, 8 Old Oak Lane, Niantic, Connecticut, USA.

The counterfeiting of pharmaceutical products threatens consumer confidence and product efficacy, as well as the economic well being of pharmaceutical companies. It is estimated that 50-70% of the drugs in some developing countries are counterfeit. The World Health Organisation and some industry analysts estimate that approximately 5-8% of drug products shipped to the US are counterfeit, unapproved or substandard. At the Pharmaceutical Anti-Counterfeiting Meeting in Washington DC in October 2001 it was stated that the proceeds from pharmaceutical and other product diversion significantly underwrote the terrorist events of September 11th 2001.

Recent studies of the natural stable isotopic fingerprints of pharmaceutical products indicates a new highly specific method for product monitoring that will allow mitigation of counterfeiting, countertrading, vicarious liability and theft.

Stable isotopes are non-radioactive elements. The heavy and light pairs of isotopes of one element can be used to form a 'tracer'. For example, 'heavy' carbon and 'light' carbon can be used to form the tracer $^{13}\text{C}/^{12}\text{C}$. Stable isotopes are measured using an isotope ratio mass spectrometer. Dual inlet instruments require the sample to be converted to a gas prior to introduction into the instrument. The use of an elemental analyser mass spectrometer (EAMS) allows the sample to be converted to a gas on-line prior to introduction into the mass spectrometer. Stable isotope ratios are expressed in units of delta:

$$\delta^{13}\text{C} (\text{‰}) = \frac{(R_{\text{smp}} - 1)}{R_{\text{std}}} \times 1000$$

where, $R_{\text{smp}} = ^{13}\text{C}/^{12}\text{C}$ of a sample, and
 $R_{\text{std}} = ^{13}\text{C}/^{12}\text{C}$ of an international standard (e.g. VPDB)

(That is, the δ -notation represents a part-per-thousand difference from a standard).

Molecular Isotope Technologies and Micromass carried out a study on analgesic isotopes. Analysis of the oxygen- ($\delta^{18}\text{O}$), carbon- ($\delta^{13}\text{C}$) and hydrogen- ($\delta^2\text{H}$) isotopic compositions of four commercially available analgesic drugs, acetaminophen and aspirin, strongly indicated that the majority of batches have their own characteristic stable isotopic fingerprints. A study with the US Food and Drug Administration (FDA) of four isotopes (including nitrogen, $\delta^{15}\text{N}$) of four active pharmaceutical ingredients -hydrocortisone, quinine HCl, tryptophan and tropicamide produced similar results.

The presentation summarised that isotopic fingerprinting is a highly specific means of characterising industrial (pharmaceutical) materials and thereby identifying and protecting these materials. All sample suites of drug substances measured showed significant dynamic range variations for the isotopes measured. Batch series variations in δ -values were both continuous and episodic due to different raw

materials and variable synthesis conditions. Isotopic crossplots showed that the combined dynamic ranges of these analgesic products yield approximately 5×10^5 possibilities or 'combinations'. The present Isotopic Product Authenticity work on ethical pharmaceuticals focuses on multi-isotopic identification of batch produced products and is subject to pending patents in at least the G8 countries and Australia.

In discussion it was speculated that spiking seen in the batch isotope records may be due to companies purchasing products from one another to market as their own. It may also be due to different production facilities within a company. The use of a chromatographic separation technique such as HPLC will allow for compound specific isotope ratio analysis.

The Use of CF-IRMS as a Tool in Forensic Soil Analysis.

Debra Croft, Forensic Geoscience Unit, Royal Holloway University of London, Egham, Surrey, TW20 0EX, UK.

Forensic geoscience can be applied to a number of serious crime investigations, such as murder, armed robbery, rape, drug smuggling, terrorism, arson and hit and run traffic accidents. The main types of geological evidence are mud, sand, gravel and rock, dusts, biological material, mineral particles, organic particles and anthropogenic particles. Samples of geological materials from restricted geographical areas often possess highly singular properties and assemblages of constituents. Footwear, clothing, skin/fingernails/hair/cavities, bags, boxes, vehicles, implements (spades, spoons, ropes etc) and household items (carpets, furniture etc) are all potential sources of geological evidence.

Initial studies on the use of CF-IRMS for soil analysis analysed triplicates of ten soil samples drawn from cases all over the UK. $\delta^{13}\text{C}$ values ranged from -20.3 to -28.3% . Studies continued with investigation into primary transfer of soil onto five different pairs of footwear and four implements. Samples were analysed for ^{13}C and ^{15}N . The carbon results showed that for three of the soils studied there was no statistical difference between the soil surface and the footwear samples for isotopic ratio and carbon content. One of the soil types showed significantly different results, possibly due to complex fractionation due to clay content (a river estuarine mud). The nitrogen results showed no statistical difference between the soil surface and the footwear samples for isotopic ratio or nitrogen content for all four soil types. The studies with the implements showed no statistically significant difference between profile means and implements means for isotopic ratios or carbon/nitrogen content. However, there was a large spread of data for some soil types reflecting sub-surface changes. Organic rich soils gave very different values than their sub-surface samples.

Spatial and temporal variation was studied at two London locations, Tower Hamlets cemetery and Bushy Park, both geologically similar. Both locations have similar usage and were stable, i.e. no earth movement in recent, past or planned, but had contrasting vegetation. Both locations were sampled every three months, over a two-year period for Tower Hamlets and one year for Bushy Park. The surfaces were sampled to 2cm and the samples bagged and stored at a low temperature. The soil samples were sieved, dried and ground prior to analysis. The spatial data (up to a 20m scale) showed no statistically significant differences at either site for nitrogen or carbon. Contouring the data over the geographical area showed no systematic directional component. Extending the scale (to 50m and then 100m) did show significant differences in all parameters. The temporal data showed that the annual data was variable, signified by relatively high standard deviation, particularly for nitrogen. For the Bushy Park location the seasonal change was not statistically significant for nitrogen data, but was significant for percentage carbon and borderline for $\delta^{13}\text{C}$. The differences between September and the other quarters wholly explain the difference in the carbon data. At Bushy Park the nitrogen levels were very low, affecting the reliability of the data. At Tower Hamlets the seasonal change was not statistically significant for the carbon data, but was significant for the nitrogen data. The differences between March and the other quarters wholly explain the difference in the nitrogen data. Variation in the nitrogen data is thought to be as a result of the

mixed vegetation input filtering into the soil over the winter plus other factors including microbial action.

The study concluded that carbon and nitrogen data, particularly used in combination, could be diagnostic of location. Vegetation input over the annual cycle is important, with the incorporation of organic matter affecting values from season to season, and possibly over year on year cycles. Variation in simple primary transfer (e.g. shoes and surface soil) is limited and comparisons can be reliably made. Variation in multiple source primary transfer (e.g. implements used at depth) is more complex, needs careful collection and analysis of appropriate control samples.

The discussion highlighted the fact that this was another good application for IRMS and there is the potential for looking at other isotopes in addition to carbon and nitrogen (i.e. strontium). However, thought will need to be given as to where this analysis will fit in with already established techniques which may have provided a higher degree of discrimination if used for these studies.

Forensic Applications in Continuous Flow IRMS Using the Iso-Prime.

Francois Fourel, Micromass UK Ltd, Floats Road, Wyhtenshawe, Manchester, M23 9LZ, UK.

Atoms of a particular element having the same number of protons but differing number of neutrons are called isotopes. Stable IRMS is concerned with determining the isotope content of the elements C,N,O,S,H found in light gases (e.g. CO₂, N₂, N₂O, H₂, SO₂). IRMS instruments measure the relative abundance of these isotopes to a reference gas. IRMS has the potential to answer the question of where does a sample come from rather than how much is there of the sample, which can be addressed by many other techniques. The units used to express stable isotope ratios are either atom% or Delta (per mil, ‰).

There are two application of IRMS. The first is to the measurement of natural abundance, which are natural variations in the isotopic content of substances that occur due to the different physico-chemical properties of isotopes. The second is tracer levels, where a chemical containing a 'high' dose of a minor isotope is input into a system to enable a process to be followed.

There has been much development in automated systems for isotope ratio mass spectrometry. The technique has evolved from off-line preparation with single inlet, to dual inlet, EA-continuous flow, GC-continuous flow and TraceGas. Application for the technique ranges from food adulteration through to the analysis of hydrology samples. As the technique develops new applications will evolve.

There are two main ways for sample introduction in IRMS. The first, dual inlet, allows for comparison of the pure sample gas to a calibrated reference gas. The second, continuous flow, has the sample as a transient signal with the reference gas injected as a gas pulse. Continuous flow techniques offer an alternative that is faster, simpler and more cost effective than classical dual inlet sample preparation systems. GC-combustion IRMS allows separation of a mixture of components prior to introduction into the elemental analyser.

There are a number of applications for which isotope ratio analysis has already been proven. In the field of food adulteration IRMS has been used for the detection, of amongst other things, the adulteration of honey. The principle of adulteration detection is to compare characteristics of a product with those of a similar product of known origin. Plants forming sugars through the C3 pathway (beets and most fruits) do not incorporate ¹³C in the same way as plants using the C4 pathway (cane or corn). Therefore honey, which has had the addition of corn syrup, will be distinguishable from the unadulterated product.

IRMS is an IOC recognised method in doping control in sports. GC-C-IRMS is the only technique that can unambiguously distinguish between endogenous and exogenous steroid metabolites. The Micromass IsoPrime is used for testing in international competitions.

IRMS has been used to study butterfly migration patterns by relating the isotopic ratios in a butterfly's wings to the variation in rainfall across the US. Similar studies

have been carried out looking at whale migration patterns by analysing the isotopic ratios in baleen.

IRMS has been used to study drugs. It has been used to distinguish batches of ecstasy tablets and has been used to provide information on the geographical origin, synthetic pathways, manufacturing batch and mixing of heroin. It can also be used for the authentication of pharmaceutical drugs. IRMS is of particular use for this as it uses natural abundance isotopic ratio without the requirement to spike the products. This provides a unique signature that is expected to be highly variable between batches of the same drug. Using GC and IRMS there is an effective way of targeting active compounds and measuring, very precisely, their isotopic ratios. Characterisation of the ambient batch to batch stable isotopic variation of such products represents a mean by which to 'fingerprint' individual drug batches. Bar-coding products with their specific isotopic fingerprint make them very difficult/impossible to counterfeit. Active compound authentication ultimately aids control of the supply, use and disposal of drugs saving money, reputation and lives.

In summary the measurement of isotopic ratios by EA-IRMS or GC-IRMS provides a precious tool for police and justice to provide information on geographical origin, synthetic pathways, natural/synthetic origin and batch identification.

GC/C-IRMS: Potential and Pitfalls.

Dr Wolfram Meier-Augenstein, School of Life Sciences, University of Dundee, Old Medical School, Dundee, DD1 4HN, UK.

The whole earth isotope ratios were determined and fixed at planet formation. However, fractionation processes lead to subtle variations in isotopic abundance. Isotope ratio mass spectrometers can measure isotopic abundance with a high degree of accuracy and precision. For a more convenient way of expressing small variations in isotopic abundance the δ -notation has been adopted with units of parts per mil (‰). Around 100 laboratories worldwide are engaged in bulk and compound specific isotope analysis.

Gas isotope ratio mass spectrometers are single focussing magnetic sector instruments. Isotopomers are detected by a multi-collector array continuously and simultaneously. Isotopic abundance is measured with a high degree of accuracy and precision. Samples are required to be converted into simple gases i.e. CO₂, N₂, CO, H₂ and SO₂.

GC/C-IRMS instruments comprise a gas chromatograph linked to an oxidation reactor, reduction reactor and the mass spectrometer. Despite the instruments being very sensitive there are pitfalls that will hamper accurate compound specific isotope analysis. These are problems with mass discrimination (isotopic fractionation), derivatisation, non-representative isotopic calibration and insufficient peak resolution. In itself there are five sources of mass discrimination:

- Sampling and sample preparation
- Sample injection on to the GC.
- Chromatographic isotope effect.
- Non-quantitative compound conversion into CO₂, N₂ or CO.
- Peak distortion and peak overlap as a result of passage through the combustion interface.

Being aware of these pitfalls means they can be dealt with. GC/C-IRMS is particularly useful for the analysis of compounds that cannot be extracted from their matrix. Potential uses are shown below.

- | | |
|---|--|
| ▪ Alcohol, sugar, organic acids, amino acids. | ▪ Food authenticity, source of ethanol, geographic origin. |
| ▪ Alkaloids and derivatives. | ▪ Origin and source of narcotic drugs. |
| ▪ Nitrogenous organic compounds. | ▪ Origin and source of organic explosives. |
| ▪ Terpenoids | ▪ Origin of flavours and perfumes. |
| ▪ Steroids | ▪ Doping control in athletic and sports. |
| ▪ Hydrocarbons and PAH's. | ▪ Maturity of oil / gas, environmental control. |

- Hydrocarbons, organic solvents and co-solvents.
- Arson investigation, origin / manufacture, batch information.

The use of GC[-MS]/C-IRMS allows for identification of the GC peaks plus isotopic information. The use of ion trap/IRMS allows for the combination of isotopic analysis with simultaneous identification of sample constituents thus avoiding any ambiguity in peak identity. Due to the very small sample sizes used ion trap MS is required. This is a highly cost effective method of analysis and has many potential applications including the forensic analysis of explosives, drugs and accelerants.

NITE-CRIME Thematic Network: Natural Isotopes and Trace Elements in Criminalistics and Environmental Forensics.

Dr Jurian Hoogewerff, Mass Spectrometry Team, Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, UK.

The NITE-CRIME EU Thematic Network aims to assist in the Fight Against Fraud and Crime (EU 5th Framework). The enormous material and immaterial damage caused by both fraud and crime implicates that each step towards prevention and/or prosecution can be regarded as both economically and socially beneficial, both on a European and on a global scale. The co-operation in the NITE-CRIME Network between the leading forensic institutions is unique in its kind. The Network disseminates its findings through publications in peer reviewed journals, contributions to conferences and dedicated workshops.

The requirement to uniquely characterise and compare physical evidence from fraud and environmental casework and crime scenes on an international scale is a major task in forensic science. The main target of the network therefore is to develop, harmonise and validate methods for analysing trace elements and isotopes in forensic materials. New developments in instrumentation like Laser Ablation combined with Quadrupole, Time of Flight, sector or Multi-Collector ICP-MS have created exciting possibilities for the routine 'non-destructive' isotope and trace element analysis of small and valuable specimens. Although there is an emphasis on laser ablation techniques within the network, for each commodity investigated the optimum trace element or isotope technique is being evaluated. The work program of the network at present includes float glass, bullets, hair and nails, steel, tape, marble, sugar, bovine serum and MDMA.

The Stable Isotope Network (FIRMS) developed by the University of Reading and the Forensic Explosives Laboratory complements the work of the NITE-CRIME network. Members of the NITE-CRIME Network could be used to build on FIRMS membership. It was agreed that a sharing of information / problems between the two networks would be beneficial.

Discussion Session - The Way Forward.

Chair: Sean Doyle, Forensic Explosives Laboratory, Dstl, Fort Halstead, Sevenoaks, Kent TN14 7BP UK.

The final session of the conference aimed to find a way forward for the network and to take forward ideas generated over the two days. The next stage for the network will be to:

- Publish the conference proceedings
- Produce a quarterly newsletter
- Establish and maintain an on-line forum
- Provide a workshop for the key stakeholders within 6 months of the conference
- Produce a strategy document
- Identify and facilitate research priorities
- Secure funding for a second conference in year 4
- Produce the final EPSRC report.

It was decided that the formation of working groups was the best way to achieve this. Initially three working groups would be formed. As work has already been carried out in the fields of explosives and drugs analysis these would be made into individual working groups. The third group would cover general forensic science, although it was recognised that eventually there will be scope for further individual working groups to cover, for example, soils, glass, paint. It is envisaged that each working group will have representatives from forensic scientists, the police community, academia and instrument manufactures. A number of individuals were identified at the meeting to initially form each group.

It is aimed that a workshop will be held within six months of the conference by which time the working groups would have been asked to consider a number of points relating to the development of IRMS in the fields of forensic science and that a strategy document would have been produced by each group.

It is also aimed that a quarterly newsletter is produced and that an on-line forum be developed. Assistance from the delegates was requested for these tasks, as well as assistance with the workshop facilitation.

In the closing statements thanks were given to all those involved with the conference and network organisation and to all the delegates for giving their support.

Those interested in joining the network should contact Sean Doyle, Forensic Explosives Laboratory, Dstl, Fort Halstead, Sevenoaks, Kent, TN14 7BP spdoyle@dstl.gov.uk.