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Stable isotope analysis of white paints and likelihood ratios

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Abstract

Architectural paints are commonly found as trace evidence at scenes of crime. Currently the most widely used technique for the analysis of architectural paints is Fourier Transformed Infra-Red Spectroscopy (FTIR). There are, however, limitations to the forensic analysis of white paints, and the ability to discriminate between samples.

Isotope ratio mass spectrometry (IRMS) has been investigated as a potential tool for the analysis of architectural white paints, where no preparation of samples prior to analysis is required. When stable isotope profiles (SIPs) are compared, there appears to be no relationship between paints from the same manufacturer, or between paints of the same type. Unlike existing techniques, IRMS does not differentiate resin samples solely on the basis of modifier or oil-type, but exploits additional factors linked to samples such as geo-location where oils added to alkyd formulations were grown. In combination with the use of likelihood ratios, IRMS shows potential, with a false positive rate of 2.6% from a total of 1275 comparisons.

Introduction

Architectural paints are a common type of trace evidence found at scenes of crime, especially in cases of attempted break-ins. Several methods are currently used within the forensic laboratory to identify paints as laid out in the SWGMAT document [1] produced by the Federal Bureau of Investigation. Methods include Infra Red spectroscopy and pyrolysis Gas ChromatographyMass Spectrometry, for obtaining information regarding binder polymers and organic pigments. More recently laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) has been used for the analysis of trace metals contained within automotive paints with success [2].

Here IRMS is being investigated for potential use for the analysis of architectural paints. Paints are composed of pigment, a binder, liquid and additives. The liquid allows the application of the paint; the pigment provides the colour to the paint, while the additives are used to give the paint specific properties required by the manufacturer. Finally the binder is a partly-polymeric material which dries to form a polymer which holds the constituent parts of the paint to the surface [3-5]. The binder is very important and affects almost all of the properties of the coating including adhesion, resistance properties and application properties. The binder is chosen by the role the paint has to perform. IRMS will be used to analyse ^{13}C , ^{18}O and ^2H isotopic composition of the paint originating from the organic components of the formulation, primarily the binder.

The calculation of a likelihood ratio is a commonly used measure of evidential strength in forensic science, and here, will be applied to the SIP results for 51 architectural white paint samples. The application of likelihood ratio to a variety of evidential forms has been extensively described in the literature. For general information see Aitken et al [6], and for DNA, Balding [7] and Buckleton [8].

Method

51 white paints were purchased from a variety of outlets in the UK and Ireland (Table 1). Samples were applied to cleaned glass microscope slides and left to dry away from direct sunlight for approximately three years prior to sampling. The three year period was a matter of circumstance since the Forensic Service Northern Ireland (FSNI) who provided the paints samples had these paints for three years. In addition, FSNI had established that no chemical change was occurring after that period of time. Samples were removed for analysis using a pristine scalpel and weighed into capsules for analysis. Samples for ^{13}C analysis were weighed at approximately 0.4mg into tin capsules and all samples for

¹⁸O and ²H were weighed into silver capsules at approximately 0.2mg.

IRMS Analysis

δ -Notation of stable isotope abundance

The natural abundance of stable isotopes is not a fixed constant but displays a considerable, yet subtle, degree of variation. Looking at ¹³C for example, the variation on the natural abundance of ¹³C can be as high as 0.1 atom%. This wide range reflects the varying degree of mass discrimination associated with the different pathways of carbon assimilation, CO₂ fixation or (bio-)chemical transformation of organic compounds. To give an example ¹³C isotopically speaking, palmitic acid present in olive oil is not the same as palmitic acid present in corn oil (maize oil) even though they are chemically indistinguishable giving rise to e.g. the same mass spectra. Depending on plant source this long chain fatty acid shows different ¹³C isotopic composition and can be distinguished on that basis with the typical difference being of the order of 0.0178atom%. Since these differences in isotopic composition are so minute on the atom% scale, the δ -notation has been adopted so to more conveniently express relative isotope abundance values.

$$\delta = \frac{1000(R_{\text{sample}} - R_{\text{strd}})}{R_{\text{strd}}} \quad (1)$$

In this equation, R_{sample} is the measured isotope ratio of the heavier isotope over the lighter (e.g. ¹³C/¹²C) for the sample and R_{strd} is the measured isotope ratio for the corresponding international reference material (e.g. VPDB in the case of ¹³C). International reference materials for stable isotope analysis are administered, controlled and issued by the International Atomic Energy Agency (IAEA, Vienna, Austria). In this notation, the aforementioned difference of 0.0178atom% corresponds to a difference in $\delta^{13}\text{C}$ -value of 16.3‰.

Bulk ^{13}C isotope analysis

Carbon isotopic measurements were performed using an automated nitrogen-carbon elemental analyser (ANCA) coupled to an automated breath carbon analyser (ABCA) IRMS (SerCon Ltd, Crewe, UK). 0.4mg of sample was weighed into tin capsules (Elemental Microanalysis, Devon, UK) and introduced via a solid autosampler. The elemental analyser (EA) reactor tubes were comprised of two quartz glass tubes filled with chromium(III) oxide and copper oxide, held at 1000C for combustion and for reduction, reduced copper, held at 600C. All consumables were purchased from SerCon Ltd (Crewe, UK). A post-reactor gas chromatography (GC) column was kept at 80C for separation of evolved N_2 and CO_2 . The data was processed using proprietary software Calisto (SerCon Ltd, Crewe, UK). Measured $^{13}\text{C}/^{12}\text{C}$ isotope ratios are expressed in the δ notation [‰], relative to the international standard Vienna PeeDee Belemnite (VPDB).

0.1 Isotopic Calibration and Quality control of EA-IRMS measurement

Two certified standards of known isotopic composition were used during the analysis of samples; leucine ($\delta^{13}\text{C}$ VPDB=-30.52‰) and glycine ($\delta^{13}\text{C}$ VPDB=-45.54‰). At regular intervals, system performance was monitored by running these two standards against an international reference material obtained from the International Atomic Energy Agency (IAEA, Vienna), namely sucrose IAEA CH-6 ($\delta^{13}\text{C}$ VPDB=-10.4‰). Raw data obtained were blank corrected and calibrated against the REF samples by the proprietary instrument software. If necessary, δ -values were drift corrected according to the deviation of measured δ -values from known δ -values of the quality controls [9-12].

Bulk $^2\text{H}/^{18}\text{O}$ isotope analysis

A TC/EA coupled to a DeltaPlus XL isotope ratio mass spectrometer via a ConFlo III Interface (all Thermo-Fisher, Bremen, Germany) was used for ^2H and ^{18}O isotope analysis of samples. Typically, 0.2mg of ground sample was weighed into silver capsules and introduced by means of a Costech Zero-Blank autosampler (Pelican Scientific Ltd, Alford, UK). The reactor tube comprised of a self-packed AlsintTM ceramic tube and a glassy carbon tube filled with glassy carbon granules, silver and quartz wool (SerCon Ltd, Crewe, UK), and was kept at 1450C. The post-reactor GC molecular sieve column was maintained at 85C. Data were processed using proprietary software, Isodat NT version 2.0 (Thermo-Fisher, Bremen, Germany). Measured $2\text{H}/\text{H}$ and $18\text{O}/16\text{O}$ isotope ratios are expressed as d-values in [‰] relative to VSMOW.

Isotopic Calibration and Quality control of TC/EA-IRMS measurement

The working reference gas, H_2 (BOC, Crawley, West Sussex, UK) was calibrated against VSMOW using international reference materials, IAEA-CH-7 polyethylene ($\text{d}2\text{H VSMOW} = 100.3\text{‰}$), VSMOW ($\delta^2\text{H VSMOW} = 0\text{‰}$) and SLAP ($\delta^2\text{H VSMOW} = 428\text{‰}$) as well as a laboratory certified house standard coumarin ($\delta^2\text{H VSMOW} = +62.56\text{‰}$). The H3+ factor was determined and checked on a regular basis on reference gas pulses of different signal size. Two certified standards of known isotopic composition were used to quality control samples, coumarin ($\delta^{18}\text{O VSMOW}=15.83\text{‰}$, $\delta^2\text{H VSMOW}=62.56\text{‰}$) and glucose penta-acetate ($\delta^{18}\text{O VSMOW}=23.94\text{‰}$, $\delta^2\text{H VSMOW}=-98.48\text{‰}$), which have been calibrated against internationally recognised reference materials by Iso-Analytical (Sandbach, Cheshire, UK). Polyethylene (IAEA-CH-7) was also used for quality assurance purposes [9-12]. The principle was to use span standards which cover the range expected from the samples analysed. The CO working reference gas (Air Products, Sur-

rey, UK) was calibrated relative to VSMOW using our house standard coumarin, as well as VSMOW.

Results and discussion

The IRMS observations were compiled to form a three-variable SIP of the 51 white paint samples from δ^{13} , δ^{18} and δ^2 values. The means of the isotopic values for each of these three isotopic systems for each paint are represented in Figure 1. A range of 8‰ observed for the δ^{13} isotopic composition of all 51 paints. The majority of the paints fall within a narrow range for δ^{13} -31 to -26‰, however, some of the paints show a composition of -32‰. The δ^{18} isotopic composition shows a 10‰ for the 51 white paints, where the paints show a more uniform distribution across this 10‰. The δ^2 composition of the 48 white paints has a range from -80 to -130‰.

The SIP is determined by organic components of architectural paint formulation including the binder and other additives. There appears to be no relationship between paints from the same manufacturer (Figure 2), or between paints of the same class or colour and their SIP (Figure 3). Unlike existing techniques, IRMS does not differentiate resin samples solely on the basis of modifier or oil-type, but exploits additional factors linked to samples such as geo-location where oils added to alkyd formulations were grown.

Resins and binders may be manufactured by the paint manufacturer or sourced from a supplier. Subtle changes during the synthesis of such products will alter the SIP of the formulation, which may be due to changes in manufacture or supplier. The raw materials used for the synthesis will also have an effect, especially where natural products are used and the geographical origin of the material can alter. The other significant component of the paint containing carbon, hydrogen and oxygen is the organic solvent, or oxygen and

hydrogen in the case of water based paints, which both evaporate and leave the paint upon drying, and thus will not influence the SIP of the paint sample.

Likelihood ratios

Likelihood ratios (LRs) were calculated for the SIPs produced for the 51 white architectural paints from analysis by IRMS for the propositions of same source, and different source. The calculations followed those of Aitken & Lucy [13], in that assumptions involving multivariate normality were made for the within item distributions, and the between item distributions were modelled using kernel density methods. Co-variance estimates were weighted following Cox & Solomon [14] to allow for slight imbalances in the data. A discussion of covariance estimation is in the Appendix.

A LR is the ratio of the probabilities of observing the SIP of the control and recovered specimens from the same source, compared to the probability of the observing the SIP were they selected from differing sources from a population represented by the 51 samples. If the LR is less than one, the isotopic composition suggests that the control and recovered specimens were from different sources. The closer the LR is to zero the more it suggests the control and recovered specimens were from different sources. If a LR is greater than one it suggests the control and recovered specimens were from the same source. The greater the LR the more the observations suggest the control and recovered specimens were from the same source.

All 51 white paints were used to represent the population as a whole. The paints were compared on a pair-wise basis with the isotopic composition of each of the other paints. This method produced a total of 1275 comparisons for which the paints were known to have not been from the same source, and 51 pairs from which it is known that the paints

came from the same source. One of the paints of the pair under comparison was arbitrarily labelled control and the other as recovered for the purposes of this comparison. In a criminal case, “control” and “recovered” would represent the sample of paint recovered from the scene of a crime and the sample recovered from the suspect, respectively. Further modelling of the independence structure was considered, however a partial correlation matrix of moderate values, and only three variables, did not, in this case, provide a worthwhile decomposable model. If a greater number of variables were available, or where a pair of partial correlation values indicated some obvious independence, such a model would be useful. However, considering this data set it would hold no advantage.

Of the 1275 comparisons from paints known to originate from different sources 27 (2.12%) of the calculated likelihood ratios were greater than one (Table 2). This indicates the paint samples are from the same source when in fact it is known the paints were from different sources. This would indicate a false positive rate of about 2%. However, it should be noted that 11 of those 27 gave likelihood ratios of less than 11. A likelihood ratio of this magnitude would provide very weak support [15] for the proposition that the two paints shared a source, and a more realistic false positive rate might be considered to be about 1.3% for which strong false positive evidence existed, and which might actually mislead investigators.

The calculated likelihood ratios for those comparisons where it was known the samples came from the same source, are shown in column two of Table 2. In this case all three replicate observations from each paint were used as both “control” and “recovered” samples. The likelihood ratios thus calculated are probably unrealistically high as the control and recovered samples matched exactly, and this is reflected in the false negative rate of zero percent. This would not be expected in cases where it was genuinely uncertain whether two specimens of paint shared the same origin.

To give a more realistic impression of the performance of IRMS observations in situations where two paint specimens were indeed from the same source, a second experiment was conducted using the first two replicates from each paint as the control item, and the second two replicates from each paint as the recovered item. In this experiment the simulated control and recovered items shared only a single observation. The likelihood ratios resulting from this experiment are given in column three of Table 2.

For this second experiment three likelihood ratios, those for items 41, 45 and 46, could not be calculated as too few replicates existed. From this second experiment only a single ($\approx 2\%$) likelihood ratio proved to be less than one. If only strong misleading evidence is counted then this would reduce to a false negative rate of zero.

Conclusion

Likelihood ratios have not been previously applied to the observations produced from IRMS analysis. The combination of the SIPs from the IRMS analysis of 51 white paints with likelihood ratios used as the measure of evidential worth show considerable forensic potential.

Using 51 white architectural paints as the population, and pair-wise comparisons to produce 1275 comparisons, only 27 ($\approx 2\%$) of the pair-wise comparisons produce false positives, a similar false negative rate has been found. The ability to distinguish samples of a common house-hold product such as white architectural paint by applying likelihood ratio analysis to stable isotope abundance data of 2, 13, and 18 shows great promise for stable isotope analysis as forensic tool. That being said, the results presented here also indicate that the discriminatory power of this approach would improve if stable isotope

data would be used in combination with data from other analytical techniques.

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Tables

Table 1 - list of all paints examined is omitted for this version as it is large

Table 2: Percentage distributions for the likelihood ratios from each comparison made. There are 1275 comparisons between paint specimens known to originate from different sources. The percentage likelihood ratios for these are in the column “different”. There are 51 comparisons from paint specimens known to originate from the same source. All three replicates made for each specimen of paint were used to calculate the likelihood ratios comprising the column labelled “all reps”. A second experiment using the first two replicates from each paint as the “control” item, and the second two replicates from each paint as the “recovered” item was also conducted. The percentage likelihood ratios are given in the column labelled “two reps”. Only 48 likelihood ratios could be calculated as three items had too few replicates observed for the calculations to be made. False positive (between-source comparison giving a value of the likelihood ratio greater than one) and false negative (within-source comparison giving values of the likelihood ratio less than one) rates are given for each set of comparisons.

| Likelihood ratio | different | same (all reps) | same (two reps) |
|---------------------|-----------|-----------------|-----------------|
| $\leq 10^{-6}$ | 90.04 | 0.00 | 0.00 |
| $10^{-6} - 10^{-5}$ | 1.50 | 0.00 | 0.00 |
| $10^{-5} - 10^{-4}$ | 1.25 | 0.00 | 0.00 |
| $10^{-4} - 10^{-3}$ | 1.02 | 0.00 | 0.00 |
| $10^{-3} - 10^{-2}$ | 1.41 | 0.00 | 0.00 |
| $10^{-2} - 10^{-1}$ | 1.41 | 0.00 | 0.00 |
| $10^{-1} - 10^0$ | 1.25 | 0.00 | 2.08 |
| $10^0 - 10^1$ | 0.86 | 0.00 | 2.08 |
| $10^1 - 10^2$ | 1.18 | 0.00 | 22.92 |
| $10^2 - 10^3$ | 0.08 | 58.82 | 68.75 |
| $10^3 - 10^4$ | 0.00 | 41.18 | 4.17 |
| $10^4 - 10^5$ | 0.00 | 0.00 | 0.00 |
| $10^5 - 10^6$ | 0.00 | 0.00 | 0.00 |
| $\geq 10^6$ | 0.00 | 0.00 | 0.00 |
| false +ve/-ve | 2.12 | 0.00 | 2.08 |

Table 3: Covariance matrices.

| U | | | |
|-----|--------------|-------------|---------------|
| | C13 | H2 | O18 |
| C13 | 0.0562834985 | 0.04701715 | 0.0001403351 |
| H2 | 0.0470171518 | 6.91225196 | -0.4951879449 |
| O18 | 0.0001403351 | -0.49518794 | 0.1658308949 |
| C | | | |
| | C13 | H2 | O18 |
| C13 | 1.9044336 | 5.861144 | 0.1065772 |
| H2 | 5.8611437 | 167.673606 | -15.4431741 |
| O18 | 0.1065772 | -15.443174 | 5.8304358 |
| C/U | | | |
| | C13 | H2 | O18 |
| C13 | 33.83645 | 124.65969 | 759.44797 |
| H2 | 124.65969 | 24.25745 | 31.18649 |
| O18 | 759.44797 | 31.18649 | 35.15892 |

Figures

Figure 1: The means of δ^2H , $\delta^{13}C$ and $\delta^{18}O$ observations for each of 51 samples of white paints.

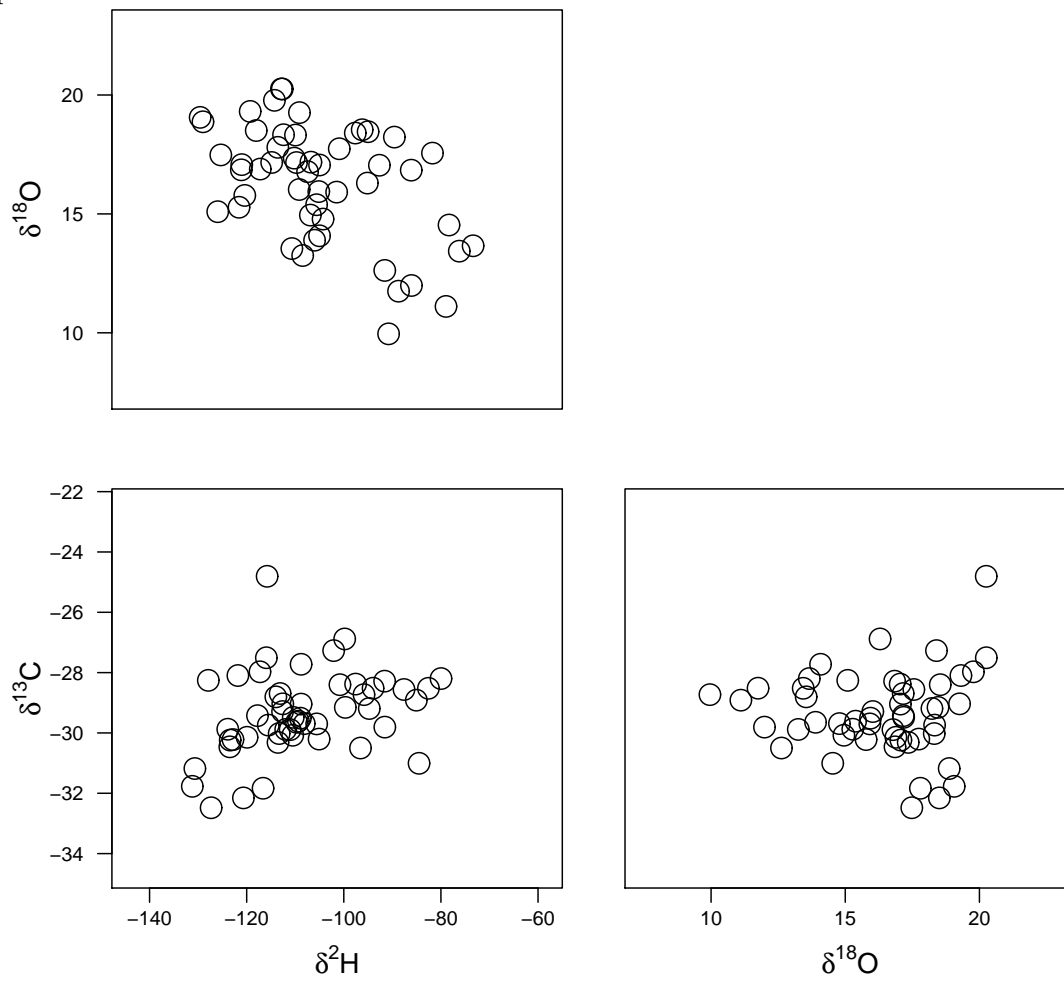


Figure 2: δ^2H , $\delta^{13}C$ and $\delta^{18}O$ observations for each of 8 makes of white paints. Represented in the sample of 51 paints are 11 makes of paint, however, only those makes with more than 6 observations have been used. The makes are represented as ellipses corresponding to 95% of the empirical distribution for that make.

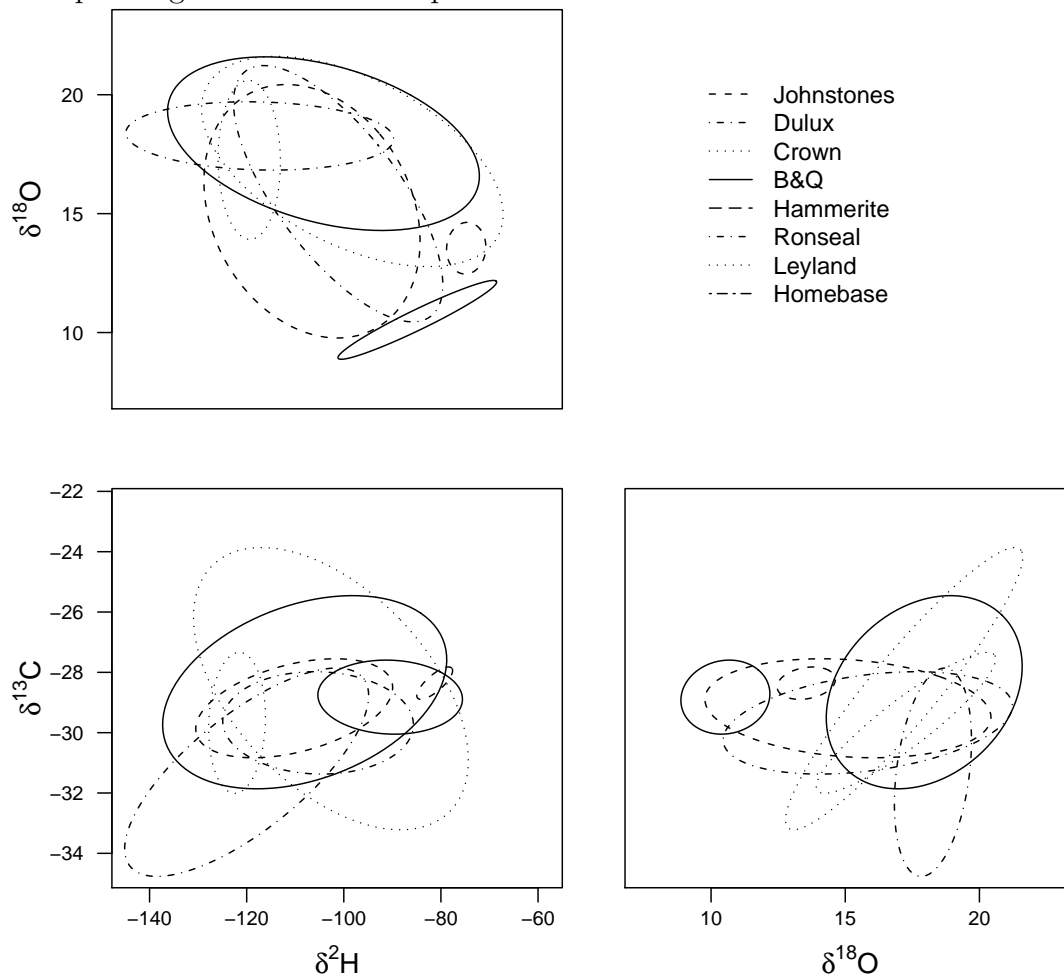
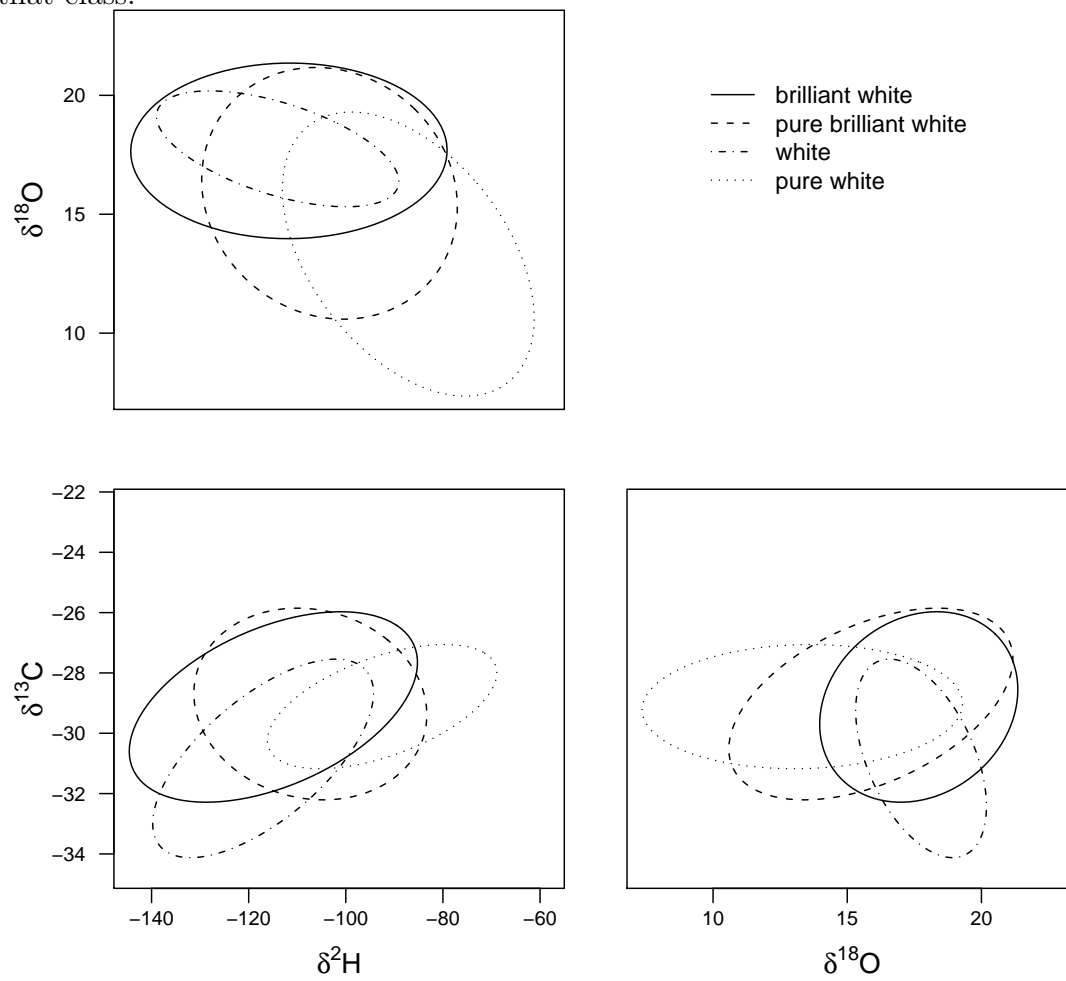


Figure 3: δ^2H , $\delta^{13}C$ and $\delta^{18}O$ observations for each of 4 classes of white paints. The classes are represented as ellipses corresponding to 95% of the empirical distribution for that class.



Covariance estimation

Suppose m white paints have been observed, such that each white paint has n_i replicate observations made upon it. The resultant matrix of observations is denoted \mathbf{x} , such that the j^{th} replicated observation upon the i^{th} item is: $x_{ij} = \{\delta^{13}\text{C}_{ij}, \delta^{2}\text{H}_{ij}, \delta^{18}\text{O}_{ij}\}$.

For datasets where imbalance is observed, that is where it is not generally true that $n_1 = n_2 = \dots n_i$, then a system of weighting, adapted from (Cox & Solomon), has been applied. For situations where conditions of balance are true, then the total number of observations is $N = nm$, where n would be the number of replicate observations made upon each item. In the more general case, outlined here, the total number of observations $N = \sum n_i$.

Lack of balance has no affect on the lowest level of covariance, so a mean may be calculated for each item:

$$\bar{x}_i = \frac{1}{n_i} \sum_j^{n_i} x_{ij}$$

However, a weighted mean is required for the mean of all items:

$$\bar{x}_{..} = \frac{1}{N} \sum_{i=1}^m \bar{x}_i n_i$$

The within item sum of squared deviations is, again, not subject to imbalance, thus:

$$S_w = \sum_{i=1}^m \sum_{j=1}^{n_i} (x_{ij} - \bar{x}_i)(x_{ij} - \bar{x}_i)^{\mathbf{T}}$$

and the covariance component estimate being made:

$$\hat{U} = \frac{S_w}{N - m}$$

The between sum of squared deviations is subject to imbalance, so takes on a weighted form:

$$S^* = \frac{1}{N} \sum_{i=1}^m n_i (\bar{x}_i - \bar{x}_{..}) (\bar{x}_i - \bar{x}_{..})^{\mathbf{T}}$$

The covariance component being:

$$\hat{U} = \frac{S^*}{m-1} - \frac{S_w}{(N^2/m) - N}$$

where the second term in the difference is derived by substituting the mean number of replicate observations per item (N/m) in for the n in the formulation given by Aitken & Lucy 2004.

Of the 51 paints examined for this paper only two sets of observations displayed any lack of balance. These had two replicate observations made rather than the three of all the other paint specimens. It is unlikely that not taking into account this order of imbalance would have any effect upon the covariance components.