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Sequential measurement of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values in archaeological bone collagen at the Scottish Universities Environmental Research Centre (SUERC): a new analytical frontier

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ABSTRACT

RATIONALE: The use of multi-isotopic analysis ($\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values) of archaeological bone collagen to assist in the interpretation of diet, movement and mobility of prehistoric populations is gradually increasing, yet many researchers have traditionally avoided investigating sulphur due to its very low concentrations (<0.3%) in mammalian collagen. For this reason, and as a consequence of analytical detection limits, sulphur is usually measured separately from carbon and nitrogen, which leads to longer analytical times and higher costs.

METHODS: A Thermo Scientific™ EA IsoLink™ IRMS system, with the ability to rapidly heat a gas-chromatography (GC) column and concentrate the sample gas online without cryo-trapping, was used at the Radiocarbon Laboratory at SUERC. Optimisation of the GC temperature and carrier gas flow rate in the elemental analyser resulted in improved signal-to-noise ratio and sensitivity for SO_2. This allowed for routine sequential N_2, CO_2 and SO_2 measurements on small samples of bone collagen.

RESULTS: Improvements in sample gas transfer to the mass spectrometer allows for sequential $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values to be measured in 1–1.5 mg samples of bone collagen. Moreover, the sensitivity and signal-to-noise ratio of the sample gas, especially SO_2, is improved, resulting in precisions of ±0.15‰ for $\delta^{15}N$ values, ±0.1‰ for $\delta^{13}C$ values and ±0.3‰ for $\delta^{34}S$ values. Previous instrumentation allowed for the analysis of ~30 unknown samples before undertaking maintenance; however, ~150 unknown samples can now be measured, meaning a 5-fold increase in sample throughput.

CONCLUSIONS: The ability to sequentially measure $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values rapidly in archaeological bone collagen is an attractive option to researchers who want to build larger, more succinct datasets for their sites of interest, at a much-reduced analytical cost and without destroying larger quantities of archaeological material.

Keywords: EA-IRMS, sequential multi-isotopic analysis, nitrogen, carbon, sulphur, archaeological bone collagen

1 INTRODUCTION

For over forty years archaeologists have utilised the stable isotope ratios of nitrogen ($\delta^{15}N$ values) and carbon ($\delta^{13}C$ values) in bone collagen of humans and animals to gain a better understanding of past diet,[1] while more recently, stable sulphur isotope ratios ($\delta^{34}S$ values) have been exploited to study the mobility and movement of populations.[2-6] Although $\delta^{34}S$ values have proven to be an invaluable additional isotopic marker for 'unpicking' diet,[7-10] the number of studies benefitting from its insight is still low due to the analytical challenges associated with producing, transferring and measuring SO_2 gas, as well as the accuracy and precision challenges that commonly accompany sulphur isotope ratio determination. While there are a small number of studies that have documented various analytical configurations to enable the sequential measurement of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values in materials such as animals, plants and sediments,[11-14] no definitive system has been widely adopted to routinely analyse samples that contain low concentrations of sulphur. There is roughly 15% nitrogen and 40% carbon in well preserved mammalian bone collagen; however, sulphur is present in concentrations of ~0.2% to 0.3%, meaning that there is approximately 2–3 μg of sulphur in 1 mg of bone collagen. Consequently, this presents a significant analytical challenge for the sequential measurement of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values by Elemental Analyser Isotope Ratio Mass Spectrometry (EA-IRMS), and it has resulted in the need to analyse one sample for $\delta^{15}N$ and $\delta^{13}C$ values (0.5–1 mg) and another larger sample (10–15 mg) for $\delta^{34}S$ values to obtain enough signal in the mass spectrometer, also known as sufficient signal-to-noise ratio, and to produce acceptable data precision.

Previous to this study, the measurement of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values in bone collagen within the Radiocarbon Laboratory at the Scottish Universities Environmental Research Centre (SUERC) was undertaken using an elemental analyser that used a constant helium carrier flow gas stream and an isothermal temperature on the GC column for gas separation. This allowed for the measurement of carbon and nitrogen isotope ratios from one sample and sulphur isotope ratios in a separate sample after a technical reconfiguration of the elemental analyser. In that configuration, and stated briefly here, $\delta^{15}N$ and $\delta^{13}C$ values were achieved by combusting ~600 μg of bone collagen, while $\delta^{34}S$ values were obtained from a separate sample, requiring technical changes and maintenance to the elemental analyser. Furthermore, approximately 10 mg of bone collagen were required to produce an acceptable signal-to-noise ratio for SO_2. The precision for these values was previously reported as ±0.3‰ for $\delta^{15}N$ values, ±0.2‰ for $\delta^{13}C$ values and ±0.6‰ for $\delta^{34}S$ values, and sample throughput was limited due to the technical changes required in the analyser.[15]

The aim of this study was to investigate if the sequential measurement of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values in bone collagen could be undertaken using the Thermo Scientific™ EA IsoLink™ IRMS system using smaller sample sizes (~1.5 mg of bone collagen), while maintaining precision at currently accepted values or improving measurement precision.

2 EXPERIMENTAL

2.1 Bone collagen preparation

Bone collagen was prepared using a modified version of the Longin method.[16-17] Briefly, sample surfaces were sanded, lightly crushed into smaller fragments, and immersed in 1 M HCl for ~24–48 h to effect demineralisation. The acid was then decanted, and samples were rinsed with ultra-pure water to remove any remaining dissociated carbonates, acid soluble contaminants and solubilised bioapatite. The gelatinous-like material was then heated gently to ~80 ˚C in ultra-pure water for ~4 h to denature and solubilise the collagen. After cooling, the solution was filtered, reduced to ~5 mL and freeze-dried.

2.2 Instrumentation and analytical configuration

A recent advance in EA-IRMS has presented the potential for rapid, accurate and precise determination of sulphur isotope ratios at concentrations less than 5 µg, while simultaneously acquiring $\delta^{15}N$ and $\delta^{13}C$ data. The EA IsoLink™ IRMS system (Thermo Fisher Scientific, Bremen, Germany) introduced two major technical improvements for elemental analysis to facilitate such measurements: the temperature of the GC column and the helium carrier gas flow rate can be optimised rapidly during sample analysis, i.e. after the sample has been combusted and is transferring through the elemental analyser. Specifically, this means that the GC column can be used either isothermally (i.e. at one set temperature) or can be rapidly heated and cooled, improving gas transfer and separation power, while sample gases are being chromatographically separated. The helium carrier gas flow rate can also be increased or decreased during the analytical process, which allows for a concentration of the analyte gas per unit volume time and means that there are more sample gas molecules being transferred to the ion source per volume time. This modification gives rise to greater sensitivity and, when combined with optimised GC column temperatures, an improved signal-to-noise ratio, resulting in better analytical precision on sample measurements. This is a significant change from previous EA-IRMS systems, which used isothermal GC and a constant helium carrier gas flow rate, and it represents a new approach in continuous-flow isotope ratio mass spectrometry.

As a consequence of these technical advances, sequential $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values of archaeological bone collagen were obtained in this study by combusting approximately 1–1.5 mg of bone collagen. Samples were weighed into a tin capsule and introduced via a MAS Plus Autosampler (Thermo Fisher Scientific), alongside a pulse of oxygen to aid combustion, to a single combustion reactor (45 cm quartz tube; from the bottom: 3 cm quartz wool, 14 cm copper wires, 2 cm quartz wool, 6 cm tungstic oxide, 1 cm quartz wool, removable quartz ash filter), which was held at 1020 ˚C. The resulting N_2, CO_2 and SO_2 gases produced in the reactor were separated using a temperature variable GC column (70–240 ˚C), and then transferred to a DELTA V™ Advantage isotope ratio mass spectrometer via a ConFlo IV Universal Interface (both from Thermo Fisher Scientific). Optimising the gas chromatography separation and helium carrier gas flow during sample analysis in the EA IsoLink™ IRMS system enabled sequential $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values of archaeological bone collagen to be obtained in approximately 10 minutes, providing sharp peak shapes, and significantly improved sensitivity

and signal-to-noise ratios for SO_2. This was achieved by optimising the timing of the increase in temperature on the GC column and the timing at which the helium carrier gas flow rate was reduced.

2.3 Bone collagen analysis

All bone collagen samples analysed in this study were calibrated against three in-house standards, which have been calibrated against USGS40 (L-glutamic acid: $\delta^{15}N_{AIR}$ = –4.52‰, $\delta^{13}C_{V\text{-}PDB}$ = –26.39‰), USGS41 (L-glutamic acid: $\delta^{15}N_{AIR}$ = 47.57‰, $\delta^{13}C_{V\text{-}PDB}$ = 37.63‰), USGS43 (Indian Human Hair: $\delta^{15}N_{AIR}$ = 8.44‰, $\delta^{13}C_{V\text{-}PDB}$ = –21.28‰, $\delta^{34}S_{V\text{-}CDT}$ = 10.46‰), IAEA-S2 (silver sulfide: $\delta^{34}S_{V\text{-}CDT}$ = 22.7‰) and IAEA-S3 (silver sulfide: $\delta^{34}S_{V\text{-}CDT}$ = –32.3‰), respectively. Samples were analysed in duplicate and had a C:N atomic ratio between 2.9 and 3.6, a C:S atomic ratio of 600 ± 300, and a N:S atomic ratio of 200 ± 100, indicating well preserved bone collagen.[18-19] A comprehensive breakdown of the data for all samples analysed in this study is given in Tables S1 and S2 (supporting information). In addition, tests were run on a bone collagen sample and IAEA-S2 to test for oxygen isotope effects on the sulphur isotopes. This was done by changing the C/S ratio of the measured sample by adding different amounts of dextrose to the same sample weight of bone collagen and IAEA-S2.

2.4 A note on sensitivity and signal-to-noise ratio

For the purposes of the proceeding discussion, we define what we mean by the word "sensitivity". In this paper, "sensitivity" is defined as:

Vs/µg of a measured element

where "Vs" equates to the total area under the integrated chromatographic peak, measured in volt seconds (Vs) and "µg" relates to the weight of an element in the sample analysed, such that it relates directly to the total area of the chromatographic peak. For the avoidance of doubt, the "µg" of the element is derived from knowing the total bulk sample weight and the percentage of the bulk sample that is related to the carbon, nitrogen and sulphur concentration therein. From this point on in the manuscript, we will simply refer to "sensitivity".

In addition, and for the avoidance of confusion over definitions, we define here signal-to-noise ratio in the context of EA-IRMS and our discussion. The signal is defined as the change in the instrument response resulting from the introduction of sample gas (analyte signal) and is measured from the middle of the baseline to the top of the chromatographic peak. The noise is defined as the variability in the instrument background signal, also known as the baseline. Therefore, the signal-to-noise ratio is defined as ratio of the analyte signal (S) to the instrument noise (N), calculated as:

$$\text{Signal-to-Noise Ratio} = S / N$$

In this manuscript, we will calculate the sensitivity, based on peak area, and signal-to-noise ratio, based on peak height, only for sulphur, as this is the primary limiting factor for the

sequential determination of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values in bone collagen, especially where samples are around 1 mg total weight. It should be noted by the reader that improvements in sensitivity and signal-to-noise ratio relate to the gas transfer from the EA to the ConFlo IV and DELTA V™ Advantage isotope ratio mass spectrometer, and do not reflect any changes to the mass spectrometer itself.

3 RESULTS AND DISCUSSION

3.1 Improved sequential NCS determination: more signal, less sample, increased productivity

Previously in EA-IRMS, it was necessary to measure $\delta^{15}N$ and $\delta^{13}C$ values from a single sample of ~0.6 mg of bone collagen (see Figure 1A), and after a technical change to the elemental analyser for a specialised setup for sulphur analysis, the $\delta^{34}S$ values would be determined on a separate bone collagen sample weighing ~10 mg or more. This analytical approach is graphically illustrated in Figure 1B, where a sample of 10.768 mg of archaeological human bone collagen was analysed for sulphur only (see Figure 2A). It is important to clarify that the EA-IRMS system relating to Figures 1B and 2A is defined by an isothermal GC column held at 90 °C and a constant helium carrier gas flow rate of 100 mL/min. This particular human bone collagen sample, which contained 0.20% sulphur, equating to 21.536 µg of sulphur in 10.786 mg of bone collagen, produced a SO_2 peak with a total area of 29.997 Vs - a sensitivity of 1.39 Vs/µg of sulphur. The same bone collagen sample was then analysed by the EA IsoLink™ IRMS system (see Figure 1C); however, only 0.996 mg of bone collagen was combusted, which contained 1.992 µg of sulphur (Figure 2B). The analysis on the EA IsoLink™ produced a SO_2 peak with an area of 0.581 Vs, equating to a sensitivity of 0.29 Vs/µg of sulphur, based on 33% of the sample gas produced in the reactor being transferred to the mass spectrometer. Given this transfer ratio, the sensitivity is accurately stated as approximately 0.90 Vs/µg of sulphur. In this example, this means that the basic technical advances in the EA IsoLink™ IRMS system, described in Section 2.2, have resulted in a sensitivity of 0.90 Vs/µg for sulphur, and while this is approximately 35% lower than previously measured (1.39 Vs/µg), it is based on the analysis of a sample 10 times smaller.

In a subsequent analysis of the same bone collagen sample, the helium carrier gas flow rate and GC column temperature were optimised to further improve the sample signal derived from the EA IsoLink™ IRMS system. By making specific, simultaneous changes to the helium carrier flow rate and to the GC column temperature, it is possible to increase the relative concentration of the sample gas transferred onto the GC column. Consequently, there is a more efficient gas transfer from the elemental analyser to the mass spectrometer, which results in an increase in ion production per unit volume time. A 1.020 mg sample, containing 2.040 µg of sulphur, was combusted, and under the optimised carrier gas and GC column temperature settings, it produced a SO_2 peak with a total area of 15.181 Vs, which equates to a sensitivity of 7.44 Vs/µg based on 80% of the sample gas being transferred to the mass spectrometer (Figure 2C). Given this transfer ratio, this means the sensitivity is accurately stated as approximately 8.93 Vs/µg of sulphur. In this first enhanced sensitivity example of the EA

IsoLink™, and focussing still on sulphur, this sensitivity is a factor of 6.42 higher than the 1.39 Vs/µg obtained after the basic technical advances, based on the analysis of a sample size 10 times smaller. With such sensitivity for sulphur, these method parameters represent an excellent approach for the sequential measurement of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values in bone collagen, without the need for further optimisation.

However, further optimisation of the helium carrier gas flow rate and GC column temperature was undertaken and after this optimisation, 0.976 mg of the same bone collagen sample, containing 1.952 µg of sulphur, was combusted to produce a SO_2 peak with a total area of 22.3 Vs. This equates to a sensitivity of 11.42 Vs/µg of sulphur and represents an increase in sensitivity by a factor of almost 13 over that of the sample that was analysed using the standard operation conditions of the EA IsoLink™ IRMS system and a factor of 8 higher than with the isothermal GC approach (Figure 2B). This was achieved without having to change the sensitivity of the mass spectrometer or the transfer of the gas in the ConFlo IV Universal Interface. A summary of the optimisation parameters for each of the tests discussed above is shown in Table 1.

With respect to the signal-to-noise ratio, the results from Figures 2A–C are summarised in Table 2, and focus specifically on sulphur, as it was the limiting factor in obtaining sequential $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values in bone collagen due to its inherent low concentration. The signal-to-noise ratio is higher for the 1.020 mg sample, based on the optimised GC column temperature and helium carrier gas flow rate, than for the 10.768 mg sample run under isothermal GC column temperatures with a constant helium carrier flow rate. This demonstrates that not only does the sensitivity improve but the signal-to-noise ratio also improves with this optimised method, even where 10 times less sample size is analysed.

Consequently, by optimising the GC column temperature and helium carrier gas flow rate, it is now possible to significantly increase sample throughput and subsequently reduce system maintenance, as a chemical reactor would previously have to be replaced after measuring the $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values in ~30 bone collagen samples, whereas ~150 bone collagen samples can now be evaluated sequentially for stable nitrogen, carbon and sulphur isotope ratios before the reactor expires. Overall, the ability to capture $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values from a single analysis of ~1 mg of bone collagen means that as well as an increase in productivity, the stable isotope facility within the SUERC Radiocarbon Laboratory has increased its annual capacity by almost 400%. Furthermore, having the capability to vary the carrier flow rate has led to a significant reduction in helium use of at least 70%.

3.2 Sequential measurement of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values in bone collagen: analytical precision

To investigate the accuracy and precision of the EA IsoLink™ IRMS system, 20 unique human and animal archaeological bone collagen samples from various sites across Europe were prepared and measured in duplicate as described in Sections 2.1 and 2.2 and the results are presented in Table 3. It is noted that the sample weight, on average, was 1.372 mg of bone collagen, which equates on average to 195 µg of N, 539 µg of C, and 2.6 µg of S (Table S1,

supporting information). Of the 20 pairs of samples, 17 had a standard deviation of ≤0.05‰ for $\delta^{15}N$ values, while 19 had a standard deviation of ≤0.05‰ for $\delta^{13}C$ values. Due to the lower concentration of sulphur in bone collagen, more variability was observed with the $\delta^{34}S$ values; however, 16 pairs of samples still had a standard deviation of ≤0.20‰. Overall, the $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values show very high precision, with most samples showing a precision of ≤0.1‰ for $\delta^{15}N$ and $\delta^{13}C$ values and ≤0.3 for $\delta^{34}S$ values, which is a significant improvement on the previously reported values of ±0.3‰ for $\delta^{15}N$, ±0.2‰ for $\delta^{13}C$ and ±0.6‰ for $\delta^{34}S$.[15]

The samples themselves are from multiple archaeological sites on different geologies across varying time periods and, thus, the isotopic results cannot be discussed in relation to one another. However, it can be noted that the lower $\delta^{13}C$ values for the UK Bronze Age humans GUsi-1 and GUsi-9 than for those from the UK medieval/post medieval period (GUsi-2, GUsi-10, GUsi-12, and GUsi-14) are probably due to the introduction of aquatic resources such as marine and freshwater fish to the diet.[20-21] GUsi-11 and GUsi-13 are both from coastal sites and this is reflected in their higher $\delta^{34}S$ values, probably due to the influence of the marine sea-spray effect.[22] The increased $\delta^{15}N$ and $\delta^{13}C$ values for GUsi-16 versus GUsi-15, both of which are from the same archaeological site, could indicate that the chicken was consuming a higher protein-based diet that may have been sourced from leftover food scraps[15] while, as expected, the cow was eating a terrestrial herbivorous diet.

3.3 Sample size and linearity

Using an Iron Age horse bone collagen sample prepared as described in Section 2.1, 7 samples of different weights (labelled in Table 4 as A–G) were analysed following the procedure outlined in Section 2.2. The $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values of GUsi-6644 were measured sequentially and showed a standard deviation of ±0.07‰ for nitrogen, ±0.06‰ for carbon and ±0.13‰ for sulphur. By plotting the peak height of the resulting gases versus their corresponding δ values (see Figures 3A–C), the linearity for NCS measurements is demonstrated, which shows that the sample size has a negligible effect on the calculated delta value for the sample measured. For N, each 1000 mV increase will produce an expected increase of 0.0126–0.0372‰ in the $\delta^{15}N$ value, with a mean increase of 0.0249‰. Similarly, for C, a 1000 mV increase will produce an expected change of 0.00964–0.0399‰ in the $\delta^{13}C$ value, with a mean increase of 0.0246‰, while for S, a 100 mV increase will produce an expected increase in the $\delta^{34}S$ value of 0.0347–0.0773‰, with a mean increase of 0.0558‰. As with all EA-IRMS analyses, linearity effects associated with total sample mass are best controlled with reproducible sample weighing to within ±5%, sample combustion and gas transfer through the EA into the mass spectrometer.

While there is a slight increased shift in the $\delta^{34}S$ value with sample size, overall the linearity is excellent within the instrument, and this is accounted for within the quoted error. This bodes well for measuring very small sample concentrations, e.g. when studying an individual's diet from early childhood through to adulthood using incremental slices of dentine, where the amount of dentine produced decreases as you move from the crown to the root of the tooth.

3.4 Negligible effect of oxygen isotopes on $\delta^{34}S$ value

Following the recommendation of Fry et al[23] and Mambelli et al,[14] tests were undertaken on two sample materials to determine the effect of oxygen isotopes on the $\delta^{34}S$ value. This was deemed important due to the number of oxidising agents in existence within elemental analysers designed for online determination of $\delta^{34}S$ values by combustion, alongside the variations evident in the sample types (organic versus inorganic) and the concentration of sulphur (high and low concentrations). Fry et al[23] provided empirical and analytical approaches for dealing with the $\delta^{34}S$ value offset resulting from varying effects of oxygen isotopes during the online conversion of sulphur to SO_2.

Here, we tested two samples to determine the effect of oxygen isotopes on the SO_2 molecule, and the subsequent $\delta^{34}S$ value, in the EA IsoLink™ IRMS system. As described in Section 2.2, our analytical set-up did not include the SiO_2 (or quartz) buffering solution proposed by Fry et al.[23] Table 5 lists the results from 3 replicate analyses of a bone collagen sample (GUsi-6900) and silver sulfide (IAEA-S2). The data show no change in the $\delta^{34}S$ value for the bone collagen and silver sulfide samples across changing C/S ratios of the sample. Consequently, no data corrections were required for the $\delta^{34}S$ values reported here.

4 CONCLUSIONS

We have demonstrated that the EA IsoLink™ IRMS system can process bone collagen samples for the determination of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values in a single sample drop using only 1–1.5 mg of material. This was achieved using the unique combination of a temperature-variable GC column and online pre-concentration of the sample gas in continuous flow mode (i.e. no form of trapping, e.g. cryo-trapping). Traditionally, given the low concentration of sulphur in bone collagen of 0.2–0.3%, samples were measured for $\delta^{15}N$ and $\delta^{13}C$ values using 0.5–1.0 mg of bone collagen, followed by a separate analysis for $\delta^{34}S$ values using 10–15 mg of bone collagen; this meant two separate analyses requiring different instrument configurations. However, the system can produce sequential $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values from small samples of bone collagen in a single analysis, which means that the overall cost per sample analysis is reduced and less maintenance is required due to a significantly higher sample throughput. Furthermore, the ability to measure all three isotope ratios using a smaller amount of sample means that a significantly smaller amount of bone, depending on preservation, is required for analysis, and hence, less archaeological material is destroyed. This is achieved without the need for corrections to the $\delta^{34}S$ values through analytical or empirical processes. In summary, access to low concentrations of carbon, nitrogen and sulphur will provide new opportunities for data collection, and in the case of sulphur, the routine nature of such measurements will enhance the use of $\delta^{34}S$ analysis in the future.

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Table 1: Summary of optimisation parameters and resulting sensitivity gains

Elemental Analyser	**Sample weight (mg)**	**GC Temp (˚C)**	**Starting$_{\text{Carrier Flow}}$ (mL/min)**	**GC$_{\text{Carrier Flow}}$ (mL/min)**	**Sensitivity (Vs/µg)**
Costech ECS 4010	10.768	90	100	n/a	1.39
EA IsoLink	0.996	70–240	180	50	0.90
EA IsoLink	1.020	70–240	180	15	8.93
EA IsoLink	0.976	70–240	180	10	11.42

Table 2**:** Signal-to-noise ratio of human bone collagen sample for *m/z* 64.

Sample Weight (mg)	**Peak Height (mV)**	**Background *m/z* 64 (mV)**	**Signal-to-noise ratio**
10.768	596	14.6	40.8
0.996	48	4.4	32.7
1.020	515	9.1	67.9

Table 3: $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values of human and animal archaeological bone collagen samples. Sample weights and µg's of N, C and S are given as averages of their duplicate measurement. *Based on commonly used UK chronologies.

Sample ID	Species	Location	Time Period*	Sample wt. (mg)	µg N	µg C	µg S	$\delta^{15}N_{AIR}$ (‰)	$\delta^{13}C_{VPDB}$ (‰)	$\delta^{34}S_{VCDT}$ (‰)
GUsi-1	Human	UK	Bronze Age	1.130	161	452	2.0	10.45 ± 0.02	–21.41 ± 0.00	7.15 ± 0.13
GUsi-2	Human	UK	Medieval	1.419	197	529	2.8	11.07 ± 0.04	–19.31 ± 0.03	6.93 ± 0.09
GUsi-3	Dog	UK	Early Medieval	1.251	201	530	2.1	11.14 ± 0.05	–19.57 ± 0.02	5.50 ± 0.09
GUsi-4	Pig	UK	Bronze Age	1.390	197	537	2.3	8.12 ± 0.01	–21.57 ± 0.01	–7.23 ± 0.27
GUsi-5	Sheep	UK	Roman	1.337	178	483	2.4	8.09 ± 0.02	–21.36 ± 0.00	13.40 ± 0.02
GUsi-6	Sheep	UK	Post Medieval	1.327	183	512	2.3	7.69 ± 0.01	–22.85 ± 0.01	7.39 ± 0.10
GUsi-7	Sheep	Montenegro	Bronze Age	1.198	165	441	2.0	4.52 ± 0.00	–20.87 ± 0.01	9.64 ± 0.17
GUsi-8	Horse	Netherlands	Medieval	1.257	175	480	2.5	7.00 ± 0.08	–22.11 ± 0.02	8.29 ± 0.02
GUsi-9	Human	UK	Bronze Age	1.171	170	465	2.0	11.49 ± 0.04	–21.66 ± 0.05	16.57 ± 0.09
GUsi-10	Human	UK	Medieval	1.345	207	571	2.9	13.34 ± 0.02	–19.69 ± 0.03	–19.27 ± 0.25
GUsi-11	Cow	UK	Early Medieval	1.369	185	514	2.6	5.36 ± 0.01	–22.07 ± 0.10	18.46 ± 0.16
GUsi-12	Human	UK	Medieval	1.260	191	530	2.6	11.11 ± 0.10	–19.52 ± 0.05	9.30 ± 0.05
GUsi-13	Sheep	Iceland	Post Medieval	1.545	219	608	2.6	3.67 ± 0.04	–20.34 ± 0.01	17.89 ± 0.18
GUsi-14	Human	UK	Post Medieval	1.378	209	567	3.0	11.88 ± 0.03	–19.64 ± 0.04	2.80 ± 0.19
GUsi-15	Cow	UK	Roman	1.513	216	603	2.6	6.93 ± 0.01	–21.47 ± 0.01	6.76 ± 0.29
GUsi-16	Chicken	UK	Roman	1.622	229	656	3.7	12.72 ± 0.02	–20.04 ± 0.04	6.52 ± 0.11
GUsi-17	Human	UK	Medieval	1.428	155	444	2.8	11.92 ± 0.10	–20.61 ± 0.04	15.93 ± 0.12
GUsi-18	Human	UK	Early Medieval	1.503	213	595	2.7	11.12 ± 0.03	–20.34 ± 0.01	11.40 ± 0.09
GUsi-19	Human	UK	Early Medieval	1.574	238	662	2.8	10.36 ± 0.04	–20.68 ± 0.04	12.40 ± 0.13
GUsi-20	Human	Georgia	Medieval	1.428	226	627	2.6	7.84 ± 0.02	–18.29 ± 0.05	1.61 ± 0.30

Table 4: $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values for an Iron Age horse; bone collagen sample weights were varied to investigate instrumentation linearity.

Sample ID	**Sample wt. (mg)**	**Ampl. *m/z* 28 (mV)**	**N_2 Area (Vs)**	**Ampl. *m/z* 44 (mV)**	**CO_2 Area (Vs)**	**Ampl. *m/z* 64 (mV)**	**SO_2 Area (Vs)**	**$\delta^{15}N_{AIR}$ (‰)**	**$\delta^{13}C_{VPDB}$ (‰)**	**$\delta^{34}S_{VCDT}$ (‰)**
GUsi-6644A	0.758	3247	87	3616	112	280	9	3.95	–21.10	7.42
GUsi-6644B	1.030	4467	116	4884	153	368	12	4.01	–21.03	7.49
GUsi-6644C	1.250	5554	140	6093	188	447	15	4.03	–20.99	7.63
GUsi-6644D	1.468	6489	163	6853	220	553	17	4.04	–21.06	7.53
GUsi-6644E	1.732	7741	197	7725	266	661	22	4.11	–21.02	7.71
GUsi-6644F	2.068	9256	232	9000	317	805	26	4.12	–20.95	7.75
GUsi-6644G	2.298	10149	262	10095	356	877	29	4.12	–20.91	7.76
S.D (1σ)								4.06 ± 0.07	–21.01 ± 0.06	7.61 ± 0.13

Table 5: Summary of tests showing negligible effect of oxygen on $\delta^{34}S$ values.

Sample ID	Sample Type	Sample wt. (mg)	Dextrose wt. (mg)	$\delta^{34}S_{VCDT}$ (‰)
GUsi-6900	Bone collagen	1.442	1.144	9.83
GUsi-6900	Bone collagen	1.478	1.626	9.11
GUsi-6900	Bone collagen	1.454	2.186	9.46
IAEA-S2	Silver sulfide	0.020	1.170	22.28
IAEA-S2	Silver sulfide	0.024	1.676	23.25
IAEA-S2	Silver sulfide	0.020	2.202	22.77

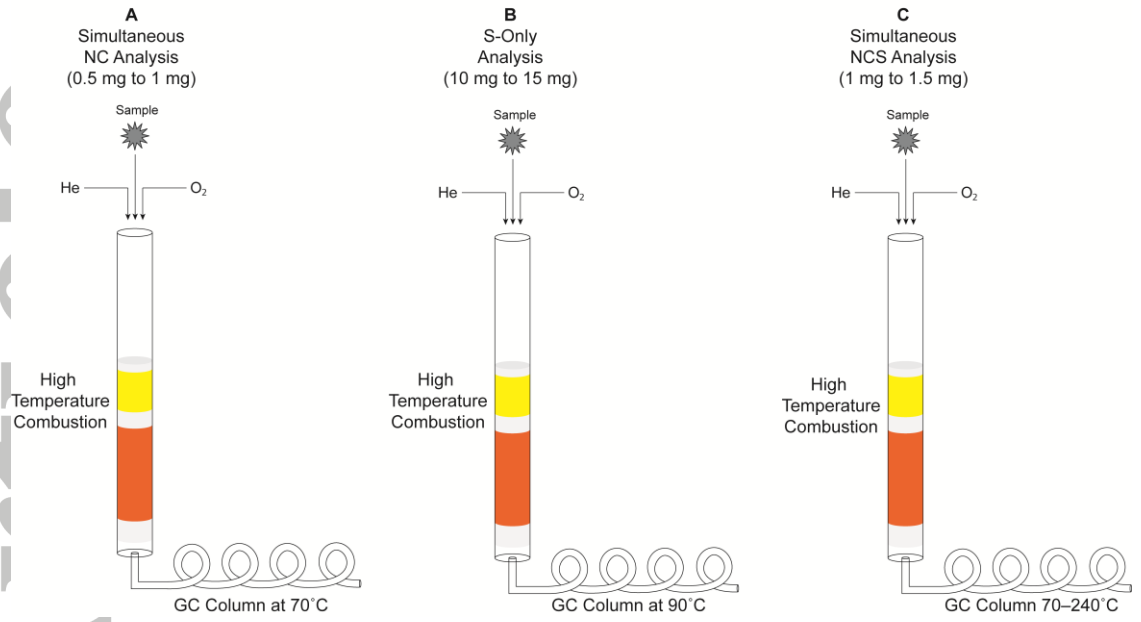

Figure 1: A comparison of previous requirements for measurement of $\delta^{15}N$ and $\delta^{13}C$ values (A), and $\delta^{34}S$ values (B), showing the need for two independent analyses on samples of different weights, and a combined determination of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values on a single sample for 1–1.5 mg of bone collagen using the EA IsoLink™ IRMS System (C).

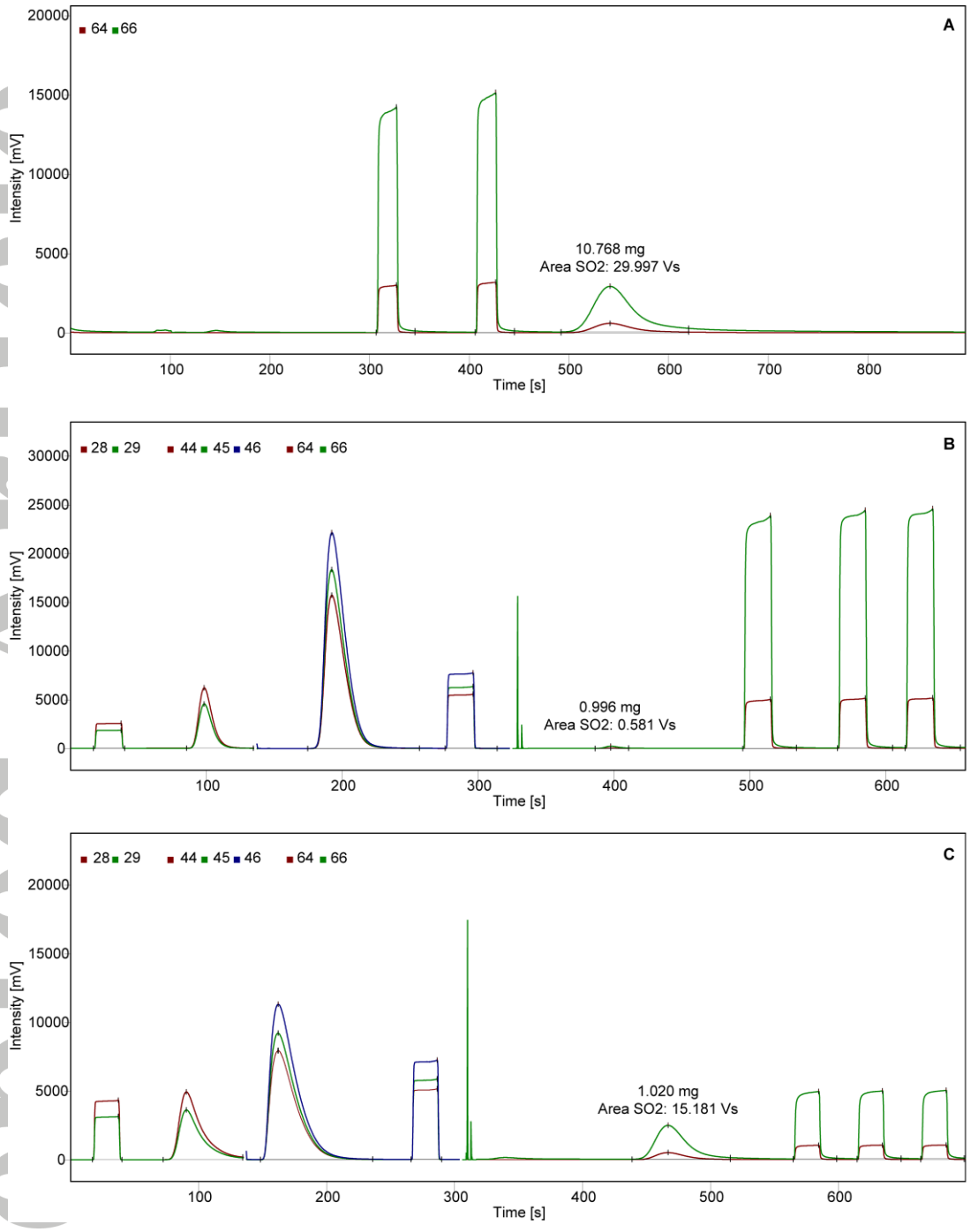

Figure 2: (A) $\delta^{34}S$ only measurement of archaeological human bone collagen (B) Sequential $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ determination of sample using EA IsoLink™ with factory settings (C) Sequential $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ measurement of sample using EA IsoLink™ with optimised GC and helium carrier flow settings.

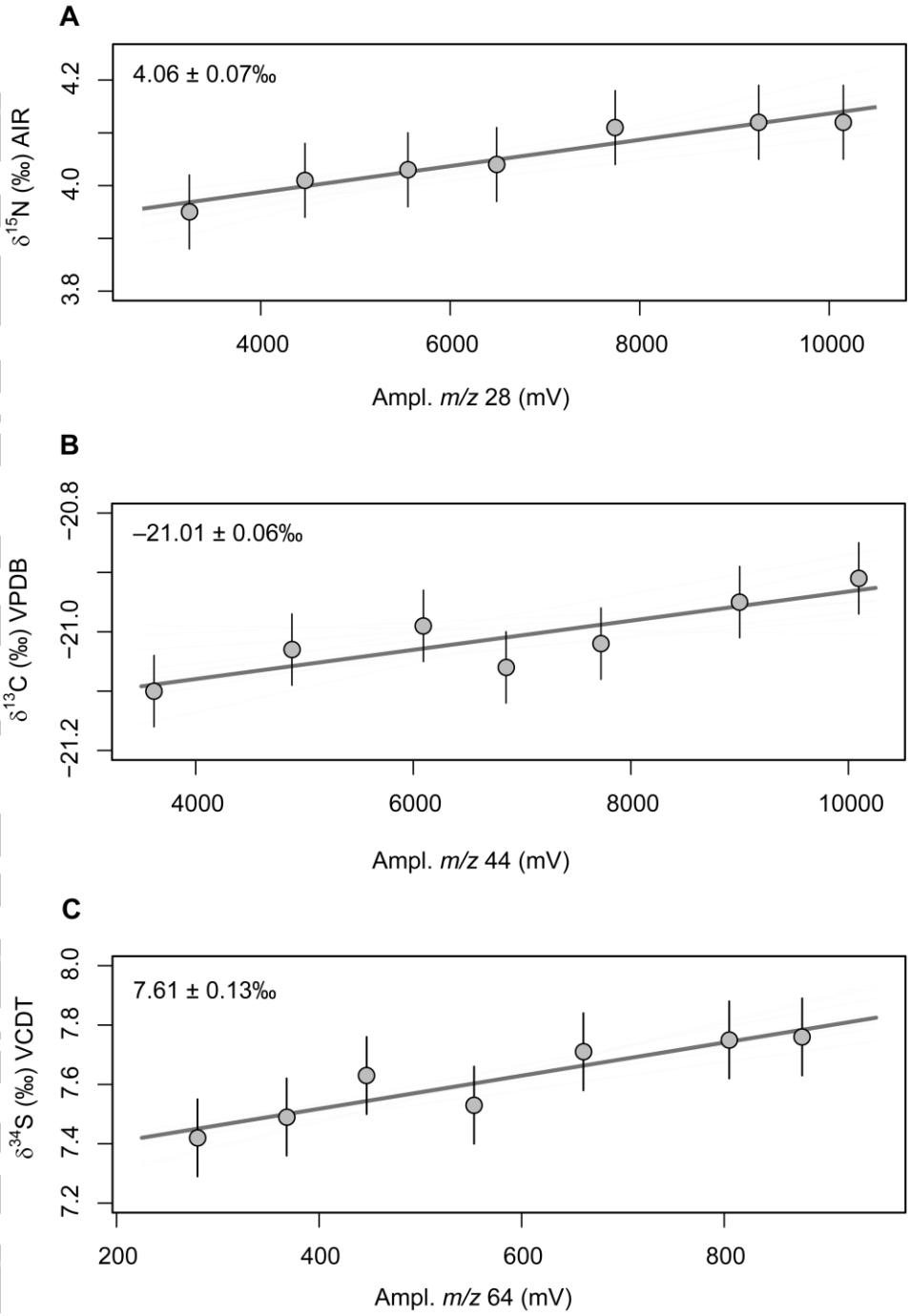

Figure 3: Plots of the peak heights for the major ion for (A) nitrogen, (B) carbon and (C) sulphur versus their corresponding δ values. With each 1000 mV increase, there is an increase of 0.0126–0.0372‰ in $\delta^{15}N$ (mean: 0.0249‰) and 0.00964–0.0399‰ in $\delta^{13}C$ (mean: 0.0246‰), and a 100 mV increase will produce an increase in $\delta^{34}S$ of 0.0347–0.0773‰ (mean: 0.0558‰).