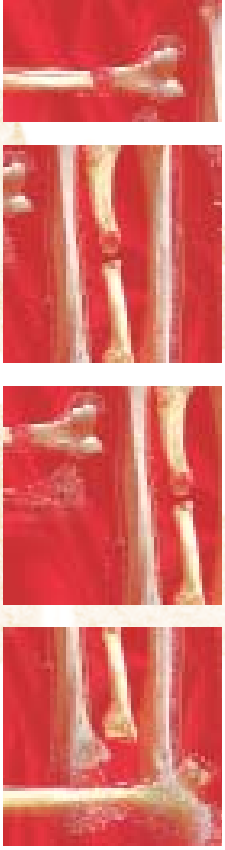

# Single-Compound Isotopic Analysis of Organic Materials in Archaeology

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**Isotopic analysis of archaeological materials provides information on age and source, but analysis of single compounds isolated from artefacts can provide more accurate measurements and additional details. This article is a review of chromatographic separations of archaeological biomolecules for their analysis using accelerator mass spectrometry or isotope ratio mass spectrometry.**

## Introduction

Isotopic analysis of archaeological materials can provide information on age, use and origin, aiding in the interpretation of the ancient societies from which the artefacts originated. Radioactive elements, because they decay at a known rate, are often used for chronology and dating of sites and objects. Several techniques exist using different elements, such as carbon, uranium, argon and potassium, which provide accurate dating from 300 years up to several million years ago (Table 1). Stable isotope ratios can be propagated through the environment, for instance from geological formations into water, or from food into body tissue, and thus these measurements are applied to studies of resource use. For example, nitrogen isotope values are carried through the food chain with regular and known changes and can be used to determine the amounts of meat and fish consumed. Some of the more common stable isotopes used in archaeological studies are listed in Table 2. This article will concentrate on studies involving lighter elements, primarily carbon and nitrogen.

While isotopic analyses are typically performed on whole artefacts or crudely isolated substances (such as bone collagen), there is growing interest in single-compound isotope measurements. Instrumentation now allows for the analysis of very small samples, and progress in separation chemistry means that this approach is feasible. Isolating a single compound can lead both to more accurate results through the elimination of contamination, as well as provide additional information through separating an isotopic signal into its constituent parts. The compounds of most interest to archaeologists are biomolecules extant in archaeological materials, namely fatty acids, proteins, amino acids and carbohydrates.

## Accelerator Mass Spectrometry (AMS)

Willard Libby won a Nobel Prize in 1960 for the development of radiocarbon dating, a technique that measures the radioactive isotope of carbon ($^{14}C$) in relation to the stable isotopes to determine the age of organic substances.[1] Traditional radiocarbon dating uses a scintillation counter and requires samples containing at least 1 g of carbon; thus in the early days of the technique the only substances dated were large pieces of less valuable material such as charcoal. However, radiocarbon dating using an accelerator mass spectrometer (Figure 1) requires samples with only 0.1–1.0 mg of carbon, allowing for dating of small or precious objects, as well as single compounds isolated from artefacts.[2] Measurement times were also reduced from weeks to hours, meaning that more samples could be analysed.[3] However, using very small sample sizes also requires that great care be taken to avoid contamination, from either the burial environment or the laboratory.

The most common materials used for AMS dating are wood, charcoal and bone, all of which go through a series of pretreatment steps prior to analysis. The exact procedure depends on the identity of the material, but commonly consists of some combination of washing with acid, base, organic solvents or bleach.[4] With bone, the collagen is isolated to remove the effects of carbon from the bone carbonate, which can exchange with the soil and render an incorrect radiocarbon date. This simple two-step process, dissolution of the bone mineral with acid followed by heating in slightly acidic water to form gelatin, results in dates that are quite accurate most of the

**Table 1:** Chronological techniques involving radioactive isotopes.

| Name of technique | Isotopes measured | Time scale |
|---|---|---|
| Radiocarbon dating | $^{14}C$ | 300–50 000 y BP |
| Uranium series | $^{238}U$, $^{230}Th$ | 1000–500 000 y BP |
| Potassium-argon | $^{40}K$, $^{40}Ar$ | 30 000–4.5 billion y BP |
| Argon-argon | $^{39}Ar$, $^{40}Ar$ | 30 000–4.5 billion y BP |

time.[5–6] However, diagenetic effects, including contamination from bacterial action during burial and Maillard reactions between autochthonous biomolecules (from within the material as opposed to its surroundings) and those from the soil can lead to erroneous dates, thus sample-specific methods are desirable for absolute removal of contaminating components.[4]

## Isotope Ratio Mass Spectrometry (IRMS)

Stable isotopes of lighter elements such as carbon, nitrogen, oxygen and hydrogen can be measured at very high precision using isotope ratio mass spectrometry (IRMS). One intensive use for this information is in the study of ancient diet, which is in turn related to important archaeological issues including subsistence strategies, technology, demographics, climate, ecology and ritual.[7–9] In archaeology, this technique is typically applied to bone collagen, but also lipids from potsherd residues and both ancient and modern hair.[10–11] Because stable isotope ratios are typically conserved within an ecosystem or transformed in recognized ways, they can be used to determine sources of archaeological matter. For instance, the stable isotope values of fatty acid residues in pots reflect the plants or animals cooked or stored in these vessels, while the signal from bone collagen relates to the average isotopic values of the foods eaten by the animal or human from whom the bone was derived. The most useful isotopes for palaeodietary studies are those of carbon and nitrogen.

Ratios of $^{13}C$ to $^{12}C$ can be used to describe the types of plants present in an ecosystem and consumed by its animal and human inhabitants.[12–14] Because the food a person or animal eats is incorporated into their body tissues, the isotopic ratios are conserved (with known modifications) through the food chain. For instance, common food crops tend to be either $C_3$ or $C_4$ plants, so named because the first photosynthetic step forms a compound containing three or four carbons, respectively. $C_4$ plants tend to grow in arid environments and include important food crops such as maize, sorghum and sugar cane, as well as grasses eaten by ruminants. They have very different biological pathways for carbon dioxide assimilation during photosynthesis and thus different isotopic compositions than $C_3$ plants, which grow in more temperate environments and include most of the fruits and vegetables that humans commonly consume. The carbon isotope ratio of bone collagen can then indicate the plant source of the food chain in which the human or animal participated. This concept has been used to examine the introduction of maize into human diets in the Americas,[14] as well as for environmental reconstruction of ancient sites in Africa.[15]

**Table 2:** Stable isotopes used in archaeological studies.

| Element | Use |
|---|---|
| Carbon ($^{13}C$) | Palaeodietary analysis ($C_3$ vs $C_4$ plant usage, marine effects) |
| Nitrogen ($^{15}N$) | Palaeodietary analysis (trophic level and marine effects) |
| Hydrogen (D) | Drinking water, climate/environment |
| Oxygen ($^{18}O$) | Drinking water, climate/environment, geographical origins |
| Sulphur ($^{34}S$) | Palaeodietary analysis |
| Strontium ($^{87}Sr$) | Geological/geographical origins |

Nitrogen isotopic signatures come from the protein that is consumed and because body nitrogen is found to be isotopically fractionated from diet nitrogen the isotope value can act as an indicator of the individual's position on the food chain.[16–17] The proportion of $^{15}N$ to $^{14}N$ increases up the food chain so carnivores have quite different isotopic signals compared with herbivores. Plotting carbon and nitrogen data together, as in Figure 2, provides an effective data analysis tool used in archaeology.[14,18] Marked on the graph are typical carbon and nitrogen values for terrestrial ecosystems, illustrating the difference between $C_3$ and $C_4$ plants and the animals that eat them. Nitrogen isotope values can also be affected by other variables, including growth, illness, starvation, pregnancy and lactation, and have been used to study both weaning[19] and dairying.[20] Climate and rainfall affect nitrogen uptake in plants and thus the nitrogen isotope values in an ecosystem.[21] Additionally, marine ecosystems tend to have very enriched carbon and nitrogen isotopic values compared with terrestrial systems.[22–23]

**Stable isotopes of lighter elements such as carbon, nitrogen, oxygen and hydrogen can be measured at very high precision using isotope ratio mass spectrometry (IRMS).**

## Single-Compound Isotope Analysis

While both AMS and IRMS measurements on bulk samples provide useful information about archaeological samples, there continues to be interest in the isolation and analysis of specific molecules from these materials. For radiocarbon dating, archaeologists are most interested in the analysis of monomers from biopolymers such as cellulose (glucose), chitin (glucosamine) and collagen (amino acids), as well as fatty acids and other non-polar compounds such as cholesterol. With radiocarbon dating of bone, isolation of 4-hydroxyproline (Hyp) from hydrolysed collagen is of most interest.[2,4–5,24] This amino acid is found in high concentration in collagen, and very rarely in other contexts, so isolation of Hyp would effectively eliminate almost all contamination from other substances in the

**Figure 1:** The accelerator mass spectrometer at Oxford (courtesy of the Oxford Radiocarbon Accelerator Unit).

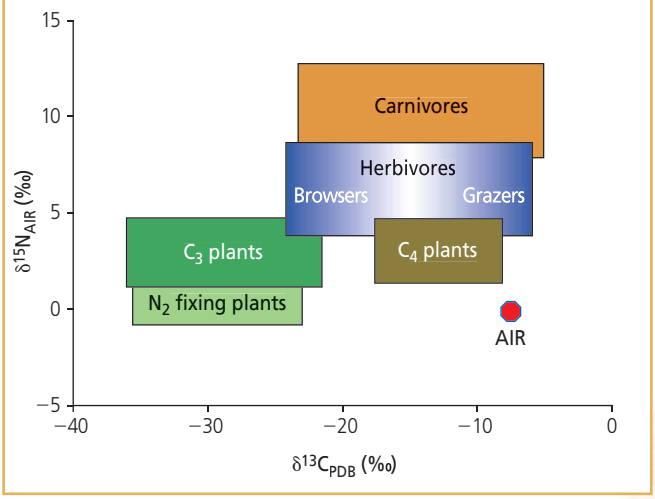
soil or laboratory. Prior experiments suggest that Hyp or proline (Pro) dates are quite accurate while other amino acids give more modern ages, probably because of contamination; the best approach is to date multiple fractions of different amino acids.[24] Because Hyp is synthesized *in vivo* from Pro, identical stable isotope or AMS data on these two compounds would suggest the absence of contamination.[24] Radiocarbon dating of monomers from chitin or cellulose allows for more accurate dates on those substances through more efficient elimination of contamination.

Stable isotope measurements on individual components of complex mixtures can, in addition to providing more accurate data, allow for the elucidation of different inputs to the isotopic signal. For instance, isotopic values of indispensable and dispensable amino acids reflect different elements of a diet. Evidence exists that carbon isotope values of D-glucosamine and its acetyl group differ, suggesting that their biochemical sources are different,[25] a fact possibly useful for examining carbon routing within insects. Multi-use pots often have complex residues and separating the different components of the residues can allow for identification of several substances that were once contained in the vessels.

In many instances it is desirable to isolate or measure several components of a system at once, making chromatography the method of choice for purification. Both gas and liquid chromatography have been used for the purification of archaeological materials. Typically the chromatography is performed on a preparative scale, and the desired compounds isolated and analysed separately. Nevertheless, there is certainly interest in hyphenated techniques and gas chromatography has been integrated with both IRMS and, recently, AMS. Integration minimizes sample handling problems and may allow these single-compound techniques to become more routine for archaeological measurements.

Archaeological separations require several considerations not normally present in chromatography method development. For instance, addition of carbon to the sample through derivatization, mobile phase components or column bleed can create errors in radiocarbon AMS or carbon stable isotope measurements and should be avoided, or, if this is not possible, added carbon must be minimized and accounted for to prevent large errors. In addition, isotopic fractionation during derivatization reactions or separation must be minimized or corrected.

**Figure 2:** Plot showing variability of carbon and nitrogen stable isotopes in terrestrial ecosystems. AIR shows values for atmospheric $N_2$ and $CO_2$.




## GC–C-IRMS

Currently, the most successful compound-specific isotope method in archaeological measurements couples gas chromatography (GC) with isotope ratio mass spectrometry, a technique alternately called isotope ratio monitoring gas chromatography–mass spectrometry (irm GC–MS) or gas chromatography–combustion-isotope ratio mass spectrometry (GC–C-IRMS). Meier-Augenstein[26] recently reviewed the development and applications of the technique. In archaeology, GC–C-IRMS has been used routinely for the analysis of lipids from potsherd residues and is becoming more popular for the analysis of polar compounds such as amino acids and carbohydrates.

Evershed et al.[27] first applied this technique to examine the carbon isotopes of organic pottery residues, soil and modern food samples and found as much as a 6‰ difference between components of the same mixture. They attributed the disparity to either the effects of cooking or variation in the primary carbon source of the residues. Evershed et al.[28] have recently published a review covering their contributions to the field, including analysis of type, structure, biological and diagenetic reactions of fatty acids, as well as their isotopic identification using GC–C-IRMS. They have been able to distinguish between ruminant and other animal fats, as well as identify dairy fats. One interesting application of GC–C-IRMS is to the analysis of cholesterol in ancient and modern bone.[29] Approximately 80% of cholesterol in the body is synthesized from dietary carbohydrates and lipids, and can give an indication of the source of these dietary components, in contrast to collagen which reflects mainly protein in the diet. In addition, the relatively rapid turnover of bone cholesterol compared with collagen allows dietary analysis at different time scales. Howland et al.[30] later used a suite of single compound analyses on bone, looking at cholesterol, fatty acids and amino acids, to examine macronutrient routing in pigs fed on six different isotopically controlled diets.

One of the first applications of GC–C-IRMS was the separation of amino acids from blood serum,[31] but because amino acids are not volatile they must be derivatized, and this can lead to large errors in measurements of carbon isotopes because the derivatization reagents inevitably contain carbon.[32–34] Silfer et al.[33] developed a derivatization technique for amino acids involving formation of the trifloroacetyl isopropyl ester derivatives of amino acids, and this is still used, though the presence of fluorine in the sample can cause problems with continuous flow IRMS methods that arise during combustion. Fractionation during derivatization can be minimized by driving the reactions to completion and ensuring that the carbon contribution from the added group is as small as possible.[32] Unfortunately, when analysing amino acids and monosaccharides large amounts of carbon must be added, up to 83% with glycine. Docherty et al.[35] demonstrated that while derivatization correction factors are reproducible and robust, errors can propagate and lead to measurement imprecision of 1.3‰ for monosaccharides and 0.8–1.4‰ for amino acids, between three and five times normal instrument error. Still, this technique can be used to examine large differences (>1.5‰) in

carbon isotope ratios between amino acids, and has been successfully used for examination of carbon isotopes of collagen amino acids from ancient human and animal bones, and modern plant tissues.[36]

If the derivatization reagents contain no nitrogen it can be a useful technique for the measurement of isotope values for that element, though several issues such as low natural abundance of nitrogen and contamination by atmospheric $N_2$ can lead to problems with reproducibility.[37] In the interest of solving some of these problems, there is great interest among archaeological scientists in the LC separation of small underivatized polar molecules such as amino acids and monosaccharides, and this will be discussed later.

## GC–AMS

Eglinton et al.[38] were the first to use preparative capillary gas chromatography to purify single compounds, in this instance from ocean sediment cores, for radiocarbon analysis. Stott et al.[39–40] applied a similar technique to direct dating of potsherds through isolation and dating of single compounds from residues. There are very few ways to directly date pottery but it is quite useful in order to correlate stylistic analyses and cultural events with absolute chronologies. Dating of pottery residues can even provide dates of sites with poor preservation because organic residues can be preserved in pottery fabric even when recovery of other organics on a site is poor. Stott et al.[40] found a significant difference between radiocarbon ages of $C_{16:0}$ and $C_{18:0}$ fatty acids from potsherds. The $C_{18:0}$ date correlated better with the known ages compared with the $C_{16:0}$ fatty acids that were consistently more modern by as much as 200 years (Figure 3). Because $C_{16:0}$ fatty acids are more abundant in the soil, they probably migrated into the sherds during burial. It was thus concluded that isolation and analysis of $C_{18:0}$ fatty acids would provide more accurate dates of organic pottery residues. In all of these instances,[38–40] fatty acids in the samples were transformed into methyl esters with radiocarbon-dead reagents. After separation they were collected, combusted, and the resulting $CO_2$ injected into the AMS source. It was noted that the compounds fractionated <5‰ during separation,[38,40] not enough to affect the radiocarbon analysis as long as entire peaks are collected.

The gas ion source at the Oxford Radiocarbon Accelerator Unit allows injection of $CO_2$ and other gaseous samples directly into the AMS at concentrations comparable to those produced by an analytical GC.[41] Recently, an integrated GC–AMS system was built, allowing samples to be separated and directly analysed.[42] Figure 4 shows a chromatogram with both $^{12}C$ and $^{14}C$ detection. The precision achieved with these sub-µg samples is approximately ±10% as determined by the statistics of detected counts. Currie et al.[3] noted that while 1% precision is needed for accurate radiocarbon dates in archaeology, only 3–10% is necessary for environmental source apportionment. So, while precision of our GC–AMS is not suitable for accurate radiocarbon dating, this system is a promising tool for examining sources of pollution and in medical studies using radiolabelled compounds.

## Liquid Chromatography

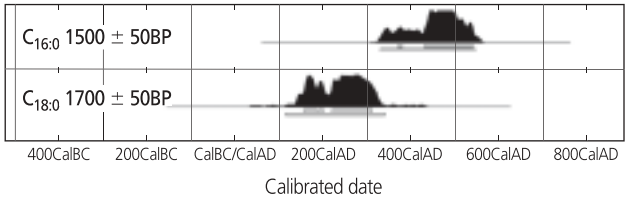
Integrated HPLC–IRMS systems are just becoming available commercially. Thermo Electron (Waltham, Massachusetts, USA) launched the Finnigan LC IsoLink at Pittcon in March 2004. Liquid chromatography (LC) has never been used on-line with AMS. More typically, preparative LC is used to isolate the compounds of interest and these are subsequently analysed off-line with IRMS or AMS. Because GC–C-IRMS works so well for non-polar compounds, LC is typically used only for separations of more polar compounds such as carbohydrates and amino acids.

**There are very few ways to directly date pottery but it is quite useful in order to correlate stylistic analyses and cultural events with absolute chronologies.**

Ion-exchange chromatography is the obvious choice for the preparative separation of polar and ionizable compounds such as glucosamine from insect chitin. Archaeological insect remains have been used as palaeotemperature and palaeoclimatic proxies, and obtaining accurate stable isotope information and radiocarbon dates aids in these studies. Schimmelmann and Deniro[25] developed an ion-exchange protocol for the purification of D-glucosamine from marine arthropod chitin to study carbon and nitrogen isotopic ratios. More recent work[43] used high-pH anion-exchange chromatography with pulsed amperometric detection and an in-line postcolumn membrane desalter to purify the same compound from insects for AMS dating, but yields of purified glucosamine after acid hydrolysis were too low to be practical for isotopic analysis. The same technique was also used for purification of glucose from wood, another common and archaeologically interesting material.[44] Unfortunately, the procedure did not provide complete removal of extraneous carbon from the enzymolysis reaction. These experiments and others[4] suggest that carbohydrates, particularly glucose, are not specific enough for sample-specific dating or isotope analysis.

Proteins, however, are more specific and often found on archaeological sites, particularly preserved bone collagen. The amino acid composition of ancient collagen is useful for determining its preservation and level of contamination, and amino acid analysis using analytical LC or GC–MS has often been put to this use. Preparative LC, however, provides purified fractions of collagenous compounds that can be further isotopically analysed. Currie et al.[3] and Stafford et al.[24] have suggested that isolating and dating a series of single amino acids from collagen can provide more information about both real age and source of contamination than simply dating bulk

**Figure 3:** Calibrated radiocarbon dates (OxCal) showing difference in age between $C_{18:0}$ and $C_{16:0}$ fatty acids from the first extraction of a Roman Mortaria sherd from the site of Stanwick. The $C_{18:0}$ age calibrates well with the actual age of the vessel, whereas the $C_{16:0}$ date is too young. Figure created with data from reference 40.



collagen or any single amino acid. Their work involved low-pressure cation-exchange LC of hydrolysed bone collagen, and provided pure fractions of up to six amino acids. A different procedure involving deamination of all of the primary amino acids, followed by extraction and separation of Hyp and Pro, provided these two secondary amino acids in sufficient purity for IRMS and AMS.[45]

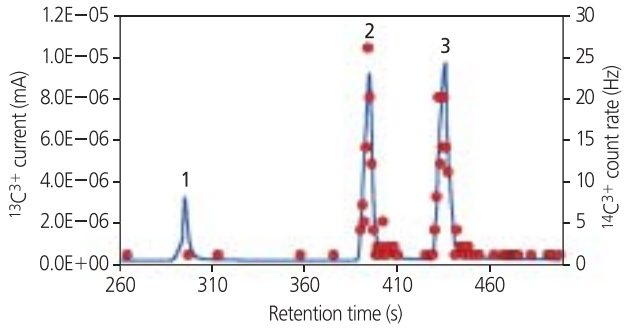
IRMS of single amino acids suggests that these compounds, particularly those that are indispensable, can have very different isotopic values compared with bulk collagen.[46] Low-pressure LC of collagen from pigs on an isotopically-controlled diet provided carbon and nitrogen isotope data on ten amino acids.[47] It was found that most dispensable amino acids were slightly enriched in heavy nitrogen compared with the bulk collagen, but threonine, an indispensable amino acid, was depleted in $^{15}N$ by about 6‰. On modern material such as this, a major advantage of chromatographic separation is the elimination of lipids, carbohydrates and other unwanted amino acids. A significant problem, however, with ion-exchange chromatography is the large amount of isotopic fractionation seen within the peaks.[47–48] Baseline separation and complete collection of the peaks is thus necessary in order to obtain accurate isotope results. In one instance,[48] only five amino acids (gly, glu, hyp, thr, ala) could be collected and analysed reliably using ion-exchange HPLC. Yet another problem with ion-exchange LC is the use of buffers that are often not volatile and must be removed by desalting of the sample prior to isotope analysis.[45, 48]

Smittenberg et al. used normal-phase chromatography for the isolation of biomarkers, particularly sterols, from marine sediments for radiocarbon dating.[49] They collected entire peaks to avoid any in-peak fractionation and were able to isolate sufficient quantities of eight lipid biomarkers for AMS analysis. However, the procedure introduced an estimated 5% of modern carbon into samples, making this technique inappropriate for high-precision work, such as that needed to produce reliable radiocarbon dates in archaeology.

Reversed-phase (RP) chromatography proved useful for the separation of peptides resulting from enzymatic cleavage of bone collagen.[50] This procedure produces a collection of tripeptides, of which Gly-Pro-Hyp and Gly-Pro-Ala are the most abundant. Gly-Pro-Hyp is much less likely than even Hyp to come from soil or laboratory contamination and thus is a useful proxy for bone collagen in AMS work. These two peptides could be isolated together and exclusively of others on a reversed-phase column with minimal fractionation. AMS analysis of this fraction provided more accurate ages than dating bulk collagen on a variety of samples, especially for very old, contaminated or degraded bones. RP-HPLC was used for the separation of singe amino acids derivatized with the non polar, fluorescent 9-fluorenylmethyl carbonate (Fmoc) group.[51] However, this process required tedious protection and deprotection of the amino acids, and excess Fmoc-OH from the derivatization reaction interfered with the separation. Six fractions could be isolated, though only four were single amino acids. Average accuracy of isotopic data from amino acid fractions were ±2.3‰ for carbon and ±1.5‰ for nitrogen. The trends in isotopic data of individual amino acids were identical to those observed using other techniques. No in-peak isotope fractionation was observed in this instance, though others have reported significant carbon isotope fractionation within peaks during RP-HPLC.[52]

Current work in our laboratory involves a two-step separation using RP followed by ion-pair (IP) separation of underivatized amino acids from bone collagen.[53] The second separation uses pentadecafluorooctanoic acid (PDFOA) as the IP reagent. This volatile compound is suitable for preparative chromatography, in which the mobile phase must be removed prior to analysis. It will also be appropriate for integration of LC with IRMS or AMS for which a volatile mobile phase is necessary. While we see isotope fractionation at a level on the order of that observed with ion-exchange HPLC, we have baseline separation of 10 of the 18 expected peaks (Figure 5), while others can be collected as pairs. We can obtain accurate isotope data on those amino acids that can be collected in their entirety.[54] This work is now being applied to the study of marine diets through the single-compound analysis of ancient bone collagen and modern hair keratin.

**Figure 4:** GC–AMS of a mixture of modern fatty acids that produce significant $^{14}C$ counts and radiocarbon dead hydrocarbon. (—) $^{12}C$, (●) $^{14}C$. Figure created with data from reference 42.




**Peaks:** 1 = hexadecane, 2 = methyl palmitate, 3 = methyl oleate.

## Conclusions

Single-compound isotope analysis of archaeological materials is an important technique to provide more accurate and useful information to archaeologists in the interpretation of ancient societies. While there is much work to be done before we have integrated, routine procedures, already single-compound isotopic techniques have provided valuable insights into the dietary habits of our ancestors. Future work will involve the integration of liquid chromatography with AMS and IRMS, and the application of high-resolution techniques to archaeological separations.

## Acknowledgments

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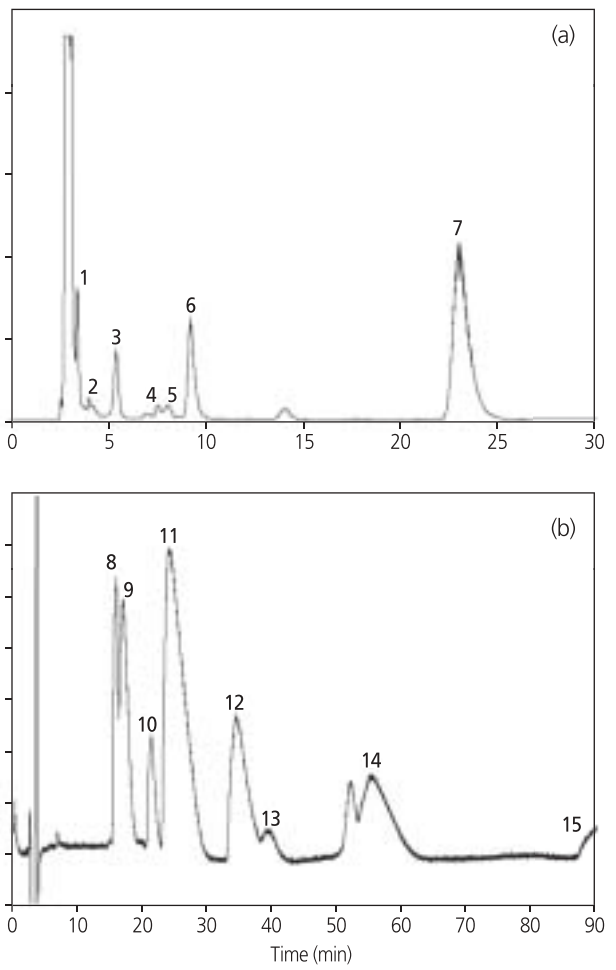
## References

1. W.F. Libby, Radiocarbon Dating (University of Chicago Press, Chicago, USA, 1952).
2. R.E.M. Hedges and J.A.J. Gowlett, *Scientific American*, 82–89 (1986).
3. L.A. Currie, et al., *Radiocarbon*, **31**, 448–463 (1989).
4. G.J. van Klinken and R.E.M. Hedges, *Radiocarbon*, **40**, 51–56 (1998).
5. R.E.M. Hedges and G.J. van Klinken, *Radiocarbon*, **34**, 279–291 (1992).
6. S. Yuan et al., *Nucl. Instr. and Meth. B*, **172**, 424–427 (2000).
7. M.J. DeNiro, *American Scientist*, **75**, 182–191 (1987).

8. S.H. Ambrose, in M. K. Sandford, Ed., *Investigations of Ancient Human Tissue* (Gordon & Breach Science Publications, New York, USA, 1993), pp. 59–130.
9. C.S. Larsen, *Bioarchaeology: Interpreting behaviour from the human skeleton* (Cambridge University Press, Cambridge, UK, 1997), Ch. 8.
10. T.C. O'Connell and R.E.M. Hedges, *J. Archaeol. Sci.*, **26**, 661–665 (1999).
11. S.A. Macko et al., *Phil Trans. R. Soc. Lond. B*, **354**, 65–76 (1999).
12. N.J. van der Merwe, *American Scientist*, **70**, 596–606 (1982).
13. T.W. Boutton, M.J. Lynott and M.P. Bumsted, *Crit. Rev. Food Sci. Nutrition*, **30**, 373–385 (1991).
14. S.H. Ambrose and L. Norr, in J. Lambert and G. Grupe, Eds, *Prehistoric Human Bone: Archaeology at the Molecular Level* (Springer-Verlag, New York, USA, 1993), pp. 1–37.
15. S.H. Ambrose and M.J. DeNiro, *Quatern. Res.*, **31**, 407–422 (1989).
16. M. Sponheimer et al., *Int. J. Osteoarchaeol.*, **13**, 80–87 (2003).
17. H. Bocherens and D. Drucker, *Int. J. Osteoarchaeol.*, **13**, 46–53 (2003).
18. S.H. Ambrose and M.J. DeNiro, *Nature*, **319**, 321–324 (1986).
19. A. Millard, in G. A. Goodfriend et al., Eds, *Perspectives in amino acid and protein geochemistry* (Oxford University Press, New York, USA, 2001), pp. 51–59.
20. A. Millard, in R. Charles and G. Bailey, Eds, *Human Ecodyamics* (Oxbow Books, Oxford, UK, 2000), pp. 134–140.


**Figure 5:** Preparative separation of hydrolysed modern cow bone collagen using preparative reversed-phase and ion-pair chromatography. Conditions: Waters Symmetry C18 19 × 150 mm, mobile phase: (a) = water, (b) = 0.5 mM PDFOA, flow-rate 5 mL/min, detection 205 nm.




**Peaks:** 1 = val, 2 = his, 3 = met, 4 = ile, 5 = leu, 6 = tyr, 7 = phe, 8 = hyp, 9 = asp, 10 = ser, 11 = gly, 12 = glu, 13 = thr, 14 = ala, 15 = pro.[54]


21. R.E. Stevens and R.E.M. Hedges, *Quatern. Sci. Rev.*, **23**, 977–991 (2004).
22. M.J. Schoeninger and M.J. DeNiro, *Geochim. Cosmochim. Acta*, **48**, 625–639 (1984).
23. M.J. Schoeninger, M.J. DeNiro and H. Tauber, *Science*, **220**, 1381–1383 (1983).
24. T.W. Stafford et al., *J. Archaeol. Sci.*, **18**, 35–72 (1991).
25. A. Schimmelman and M.J. Deniro, in R. Muzzarelli et al., Eds, *Chitin in Nature and Technology* (Plenum Press, New York, USA, 1986), pp. 357–364.
26. W. Meier-Augenstein, *J. Chromatogr. A*, **842**, 351–371 (1999).
27. R.P. Evershed et al., *Analyst*, **119**, 909–914 (1994).
28. R.P. Evershed et al., *Acc. Chem. Res.*, **35**, 660–668 (2002).
29. A.W. Stott et al., *J. Archaeol. Sci.*, **26**, 705–716 (1999).
30. M.R. Howland et al., *Int. J. Osteoarchaeol.*, **13**, 54–65 (2003).
31. D.E. Matthews and J.M. Hayes, *Anal. Chem.*, **50**, 1465–1473 (1978).
32. G. Rieley, *Analyst*, **119**, 915–919 (1994).
33. J.A. Silfer et al., *Anal. Chem.*, **63**, 370–374 (1991).
34. J.A. Silfer et al., *Org. Geochem.*, **21**, 603–609 (1994).
35. G. Docherty, V. Jones and R.P. Evershed, *Rapid Commun. Mass Spectrom.*, **15**, 730–738 (2001).
36. M.L. Fogel and N. Tuross, *J. Archaeol. Sci.*, **30**, 535–545 (2003).
37. D.A. Merritt and J.M. Hayes, *J. Am. Soc. Mass Spectrom.*, **5**, 387–397 (1994).
38. T.I. Eglinton et al., *Anal. Chem.*, **68**, 904–912 (1996).
39. A.W. Stott et al., *Radiocarbon*, **43**, 191–197 (2001).
40. A.W. Stott et al., *Anal. Chem.*, **75**, 5037–5045 (2003).
41. C. Bronk Ramsey and R.E.M. Hedges, *Radiocarbon*, **37**, 711–716 (1995).
42. C. Bronk Ramsey et al., *Radiocarbon*, in press (2004).
43. G.W.L. Hodgins et al., *Radiocarbon*, **43**, 199–208 (2001).
44. G.W.L. Hodgins et al., *Radiocarbon*, **43**, 209–215 (2001).
45. T.W. Stafford et al., *Life Sci.*, **31**, 931–938 (1982).
46. P.E. Hare and M.L.F. Estep, *Carnegie Institution of Washington Yearbook*, **82**, 410–414 (1983).
47. P.E. Hare et al., *J. Archaeol. Sci.*, **18**, 277–292 (1991).
48. G.J. van Klinken and W.G. Mook, *Radiocarbon*, **32**, 155–164 (1990).
49. R.H. Smittenberg et al., *J. Chromatogr. A*, **978**, 129–140 (2002).
50. G.J. van Klinken, A.D. Bowles and R.E.M. Hedges, *Geochim. Cosmochim. Acta*, **58**, 2543–2551 (1994).
51. T.C. O'Connell and R.E.M. Hedges, *Archaeometry*, **43**, 421–438 (2001).
52. R.J. Caimi and T.J. Brenna, *J. Chromatogr. A*, **757**, 307–310 (1997).
53. J.A. Tripp and R.E.M. Hedges, *Book of Abstracts, HTC-8, paper 17.2*, (2004).
54. J.A. Tripp and R.E.M. Hedges, *manuscript in preparation.*



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