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Practical considerations in high-precision compound-specific radiocarbon analyses: eliminating the effects of solvent and sample cross-contamination on accuracy and precision

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Abstract

Preparative capillary gas chromatography (pcGC) is widely used for the isolation of single compounds for radiocarbon determinations. While being effective at isolating compounds, there are still genuine concerns relating to contamination associated with the isolation procedure, such as incomplete removal of solvent used to recover isolated samples from the traps and cross-contamination, which can lead to erroneous ^{14}C determinations. Herein we describe new approaches to identifying and removing these two sources of contamination. First, we replaced the common "U" trap design, which requires recovery of compounds using

organic solvent, with a novel solventless trapping system (STS), consisting of a simple glass tube fitted with a glass wool plug, allowing the condensation of isolated compound in the wool and their solventless recovery by pushing the glass wool directly into a foil capsule for graphitization. With the STS trap, an average of 95.7 % of the isolated compound was recovered and contamination from column bleed was reduced. In addition, comparison of ^{14}C determinations of fatty acid methyl ester (FAME) standards determined offline to those isolated by pcGC in STS traps showed excellent reproducibility and accuracy compared to those isolated using the traditional "U" traps. Second, "cold-spots" were identified on the instrument, i.e. the termini of capillaries in the preparative unit, which can be cleaned of compounds condensed from earlier runs using a heat gun. Our new procedure, incorporating these two modifications, was tested on archaeological fat hoards, producing ^{14}C dates on isolated $C_{16:0}$ and $C_{18:0}$ fatty acids statistically consistent with the bulk dates of the archaeological material.

Introduction

Compound-specific radiocarbon analysis (CSRA) has proven to be a powerful tool in understanding C dynamics in the earth system at a mechanistic/process level by allowing the fluxes and turnover rates of individual compounds or compound classes with well-defined sources to be determined. The use of preparative capillary gas chromatography (pcGC) for the isolation of pure compounds for CSRA has been used widely in the environmental sciences since its inception by Eglinton and co-workers[1] wherein the authors demonstrated the application of the technique to radiocarbon determinations of *n*-alkanes and *n*-fatty acids. The technique has since been extended to the analysis of a range of various organic compounds in a diversity of matrices[2-6].

An important potential application of this approach is the radiocarbon dating of archeological organic residues, particularly the $C_{16:0}$ and $C_{18:0}$ fatty acids derived from animal fats which occur widely adsorbed within the clay matrix of archaeological potsherds. Although the results of initial studies[7-9] were promising, it was evident that the accuracy and precision of these determinations did not meet the high standards demanded by archaeological dating, making this perhaps the most challenging application of the technique. Any exogenous C added to the samples (especially on small sample sizes) isolated from the archaeological matrix would reduce the accuracy of ^{14}C measurements and require correction[10, 11]. Furthermore, it is critical for many archaeological applications that small chronological differences between samples (and therefore events) can be observed after calibration. The accuracy and precision that is therefore required for archaeological samples exceeds those often deemed reasonable for other CSRA applications.

Assuming sufficient analyte C is available, the major factors which can limit the achievable levels of accuracy and precision during compound-specific radiocarbon dating are: i) introduction of exogenous C during isolation by pcGC, oxidation and reduction to graphite; and ii) cross contamination or sample "carryover" between samples within the pcGC system (i.e. GC or preparative fraction collector (PFC)). The samples and standards prepared in this study were not considered to be 'small', however, for applications where only small samples can be isolated and analyzed (ca. 10-100 μg C), the assessment of blank contributions and correcting for their effects is critical and much research has been dedicated to such studies[10-13].

The matter of contamination of analytes with exogenous C incorporated during isolation of compounds by pcGC, whether from column "bleed" and/or due to residual solvent in isolated compounds after drying, has always been a matter of concern for users of the technique [1, 11, 14, 15]. In their original validation of the method, Eglinton et al.[1] used capillary columns coated

with thin films of stationary phase to limit the effects of column "bleed" arising through thermal degradation, concluding that "There is minimal ^{14}C background contamination (<<5 µg of C) introduced by the pcGC system or by the GC column". Recently, we demonstrated for the first time that although present in analytes isolated by pcGC, cyclic poly(dimethyl siloxanes) resulting from the degradation of the stationary phase of the GC column, were not present in sufficient quantities to affect high precision ^{14}C determinations[16].

The potential for organic solvents, used to manipulate analytes post-trapping, to persist after drying of compounds appears not to have been systematically investigated. The commercially available glass traps used with the Gerstel PFC consist of a coaxial glass tube fitted with a side-arm (Fig. 1A) whereby the column eluent flows down the interior channel, before flowing up the exterior and leaving the trap via a side-arm. It is this outward flow which is switched on and off by the PFC system to direct the column eluent to different traps. The isolated compounds are generally trapped when they condense in the initial few mm of the trap. They are subsequently recovered by back-flushing the trap with an organic solvent followed by removal of the solvent from the resulting solution by blowing down under a gentle stream of N_2 (Fig.1B). Since the compounds isolated by pcGC are generally lipophilic, their affinity for organic solvents could result in the incomplete removal of the organic solvents used to remove them from the traps. The low quantities of analyte trapped, combined with their often relatively high volatilities means that it is undesirable to blow analytes down too strongly or for too long, as this could result in evaporative losses. Considering that any exogenous C present at the permil level would have a significant effect on the determined radiocarbon date of an isolated compound, it is conceivable that this could represent a significant source of exogenous C in such samples. Indeed, although they were unable to identify or quantify any residual solvent using high-resolution GCMS or shifts in $\delta^{13}C$ values, in 1996 Eglinton et al.[1] noted that, "Incomplete removal of solvent prior to combustion is the

major potential source of carbon contamination" in compounds isolated by pcGC. Commonly adopted characterizations of, and corrections for, the effect of this contamination are performed using the deviation of the determined $F^{14}C$ values for standards and blank materials covering a range of sample sizes[10, 11, 15]. An obvious solution to the effects of incomplete solvent removal would be to recover isolated compounds without the use of organic solvents.

A further challenge recognized in the radiocarbon determination of organic compounds isolated by pcGC is cross-contamination between trapping sequences[15, 12, 17]. Strategies for avoiding cross-contamination involve "washing" the entire system by performing repeat injections (10x) of aliquots of the new sample, discarding the resulting isolates and replacing the traps with clean[15, 12, 17]. This practice highly is undesirable, as it constitutes loss of precious analyte especially given that isolating enough C is one of the major challenges in CSRA. Furthermore, the efficacy of this practice has not, as far as we know, been rigorously tested. It is most likely that any cross-contamination occurs as a result of compounds from earlier trapping sequences becoming condensed at 'cold spots' in the pcGC system but are re-mobilized, contaminating the subsequently isolated compound. The most likely location for this to occur is where the fused silica capillaries protrude from the heated sections of the PFC unit and enter the unheated glass traps. We propose that any residual analyte adhering to the capillaries at these locations could be removed with the application of heat.

Herein, we report a new trap design and the results of experiments conducted to: i) quantify residual transfer solvent persisting in analytes, trapped using the traditional Gerstel "U" traps, after blowing to dryness under a stream of N_2, ii) compare the sample trapping efficiency, the mass of exogenous carbon introduced, and both the accuracy of ^{14}C determinations (by comparison with off-line preparation) and the precision (scatter of ^{14}C dates) observed in replicate analyses of compounds isolated using the traditional "U" traps and our new

123 solventless trapping system (STS trap), iii) determine the degree of cross-contamination
124 between isolated analytes and its potential impact on ^{14}C determinations, and iv) assess the
125 efficacy of a simple heat gun cleaning procedure in reducing or eliminating analyte cross-
126 contamination. We show through these modifications that high precision archeological
127 calendrical dates can now be routinely obtained.

128

129 **Materials and Methods**

130 **Standards, solutions and samples**

131 All glassware was washed with Decon 90, ultrapure (18.2 MΩ.cm) MilliQ™ water and
132 acetone then pre-combusted (450°C > 5 h) before use. All solvents were of HPLC grade and
133 purchased from Rathburn (Walkerburn, UK). Deuteriated chloroform (>99.96 atom % D),
134 $C_{16:0}$ and $C_{18:0}$ FAMEs were purchased from Sigma-Aldrich (Poole, UK). The $F^{14}C$ values of
135 these standards was determined and the weighted average of the five replicates used as a
136 reference value. From these standards, a FAME standard solution was prepared in hexane,
137 containing each FAME at a concentration equivalent to 5 μg C.μL^{-1}, which is our target
138 concentration for FAMEs extracted for the pcGC isolation of archaeological samples in order
139 to obtain ca. 200 μg C. Glass wool (Assistent, Sondheim, Germany) was pre-combusted
140 (450°C > 5 h) prior to insertion in the glass tubes, which were then foil wrapped and pre-
141 combusted again before use.

142 Archeological bog butters (large quantities of fats) were selected as ideal archaeological
143 samples to demonstrate the effectiveness of this method, due to their size and hydrophobic
144 nature. These samples were recovered from peat bogs of Ireland and their lipid compositions
145 revealed them to be animal fats deriving mainly from dairy products[18, 19]. Samples were taken
146 from the center of these hoards (to avoid environmental contamination) and directly

147 combusted and graphitized. For CSRA of their lipids, FAMEs were prepared from the bog
148 butters using the method established by Correa-Ascensio and Evershed[20].

149

150 **pcGC parameters**

151 The pcGC consisted of a Hewlett Packard 5890 series II gas chromatograph coupled to a
152 Gerstel Preparative Fraction Collector by a heated transfer line. Details of the pcGC
153 parameters were previously published in Casanova *et al.*[13] and are given in supplementary
154 materials.

155 The $C_{16:0}$ FAMEs were isolated in trap 'T1' and the $C_{18:0}$ FAMEs were isolated in trap 'T2'.
156 Trap,'T0' was used as the waste trap and all column effluent passed through this trap outside
157 the trapping time-windows.

158

159 **Quantification of residual solvent after transfer from "U" traps**

160 $C_{16:0}$ and $C_{18:0}$ FAMEs were isolated by pcGC using the "U" traps, before transfer of the
161 isolated compounds into glass vials by flushing with 1 mL of dichloromethane. The isolated
162 FAME solutions were then blown to dryness under a gentle stream of N_2. The samples were
163 then re-dissolved in deuterated chloroform and transferred to 1.7 mm NMR (nuclear
164 magnetic resonance) tubes for analysis by 700 MHz microcryoprobe ^{1}H NMR as described
165 by Casanova et al.[16].

166

167 **Comparison of "U" traps and STS traps**

FAMEs standards isolated using both trap designs were quantified by GC-FID (flame ionization detector) after either flushing traps into glass vials with 1 mL of hexane ("U" traps) or by transferring the glass wool into glass vials by pushing it out using the tip of a pre-combusted glass Pasteur pipette and dissolving the trapped FAMEs in hexane (STS traps). This extract was split for both GC-FID analysis, to determine trapping efficiency, and the quantification of exogenous C by NMR. After removal of glass wool, any remaining FAMES on the inside of the STS traps were extracted with hexane to assess the partitioning of trapped compounds between the glass wool and the walls of the glass tubes. Compounds were quantified by GC-FID using the internal standard method.

Quantification of exogenous carbon

FAME standards isolated by pcGC using both trap designs were extracted and ¼ of the extract transferred to 1.7 mm NMR tubes in deuterated chloroform and analyzed using 700 MHz spectrometer equipped with microcryoprobe for the quantification of poly(dimethyl siloxanes), deriving from 'column-bleed', and screening for residual solvent and other sources of exogenous C as described previously [16].

Accuracy and precision of radiocarbon dates

Compounds isolated by pcGC (~200 μg C) were transferred to tin capsules by either (i) using the 'Russian doll' technique outlined by Stott et al.[8] in the case of the traditional "U" traps; or (ii) direct transfer of glass wool to a tin capsule in the case of the new STS traps. Samples (including the pure FAME standards) were combusted using a Vario Isotope Select elemental analyzer (EA, Elementar, Langesenbold, Germany) and the resulting CO_2 graphitized using

191 an automated graphitization system (AGE3, IonPlus, Zurich, Switzerland) using Fe (Aefa
192 Aesar, Heysam, UK) as a catalyst[21]. Graphitized samples were pressed into Al targets using a
193 pneumatic sample press (PSP, IonPlus, Zurich, Switzerland) and radiocarbon determinations
194 performed using size-matched standards and blanks on the BRIS-MICADAS system (ETH
195 Zurich, Zurich, Switzerland).

196 A mass-balance approach (outlined by Stott et al.[8]) was adopted to correct for the
197 contribution of the methyl group added in the derivatization of fatty acids, where appropriate.
198 Processing standards and blanks were prepared by performing trapping sequences whereby
199 only solvent was injected into the pcGC injector, but the trapping 'windows' were the same
200 as for real samples in order to mirror the concentrations of any exogenous C introduced by
201 this process. A total of 200 µg of C of radiocarbon-dead blank material (phthalic anhydride,
202 Sigma Aldrich) or standards (IAEA C7 and IAEA C8 oxalic acids) were added to the tin
203 capsule after the transfer of the trap contents and analyzed alongside the isolated FAMEs.

204

205 **Cross-contamination between pcGC isolated compounds**

206 Cross contamination was assessed by isolating FAMEs from the FAME standard solution
207 before injecting pure solvent onto the GC column, then the column eluent was trapped for 30s
208 at the retention times when the FAMEs would elute. This method was performed both
209 without cleaning the instrument, or after cleaning the end of the capillaries where they exit
210 the PFC using a heat gun at 300 °C under elevated He flow to evaporate any condensed
211 FAMEs. The contents of the traps were extracted and analyzed by GC-FID and any FAME
212 contamination was quantified using the internal standard method.

213

214

Results and discussion

The analysis presented below builds on our previous work[7-9, 16] and provides two significant modifications to the pcGC protocol: (i) the use of a new trap design to overcome the incomplete removal of solvent during the handling of compounds post-isolation in the "U" traps, and (ii) the use of a heat-based cleaning method for the transfer capillary system, which removes cross-contamination between trapping sequences.

Incomplete removal of solvent

After trapping, FAMEs were recovered from the "U" traps by rinsing with organic solvent, such as DCM, then 'removing' the solvent under a stream of N_2, (Fig. 1B) Despite the vials which contain the isolated compounds appearing to be solvent-free, the ^{1}H NMR spectra show a clear signal at 5.32 ppm corresponding to DCM protons (Fig. 2A). The amount of C in the final trapped FAME, which is derived from the residual DCM as a proportion of the FAME C was found to be 7.4 ‰ (or 1.8 µg of C) in trap T1 ($C_{16:0}$) and 9.3 ‰ (or 2.1 µg of C) in trap T2 ($C_{18:0}$). Since DCM is a petroleum-derived product and thus contains no radiocarbon (i.e. it is radiocarbon 'dead'), this would equate to a shift in the determined radiocarbon dates of 60 and 75 years older than the true age, respectively. These offsets would be outside the 2σ (95%) range of high-precision and typical archaeological radiocarbon determinations, where 1σ errors are in the range of 25-30 years. These results clearly demonstrate the potential for problems resulting from incomplete removal of solvent prior to radiocarbon analysis, as originally recognized by Eglinton et al.[1]. The compounds isolated above were considered to be free of solvent before NMR analysis; although determining the presence of such solvent by GC is impossible. These results emphasize the

need for using a solventless system for the recovery of compounds isolated by pcGC for radiocarbon dating for archeological applications.

Comparison of "U" and STS traps

Description of STS-trap design

The trap design for a solventless recovery (STS trap) tested herein consists of a borosilicate glass capillary (3 mm OD, 1 mm ID, 70 mm in length) containing a 10 mm glass wool plug positioned 15 mm from the top of the trap (Fig. 1C). The capillary tubes are connected to the PFC via PTFE ferrules in the same manner as the "U" traps and the silicone tube connecting the trap to the valve cluster in the PFC is attached to the bottom of the STS traps. The analyte is condensed onto the glass wool, which can be physically removed from the trap by pushing, with the tip of a pre-combusted glass Pasteur pipette, directly into a tin/foil capsule for combustion in an elemental analyzer or into a glass tube for offline combustion (Fig. 1D).

Determination of trapping efficiency

The percentages of C lost to waste (trap T0), successfully trapped and recovered in the "U" and STS traps, and in the case of the STS traps, lost to the walls of the traps, were determined by GC-FID (detailed results in supporting information Table S1). The proportion of C from the FAME which was collected in the 'waste' trap, T0, was found to be 1.3 % in the case of the "U" traps, and 1.2 % in the case of the STS traps. Any C transferred to the waste trap is likely to be a consequence of the switching of the traps during the tail of the chromatographic peaks and should therefore be independent of the trap design; our data support this as the amount of FAMEs lost in the waste is identical for both designs.

Using the STS trap, a potential source of analyte loss would be due to condensation on the internal walls of the glass tube. Determination of the proportion of C lost on the sides of the tubes in the STS traps was shown to be 3.2 ± 4.4 % (1σ). Losses of up to 12.2 % were observed for the two most extreme cases, in traps where the PFC capillary was not in contact with the glass wool. It can therefore be concluded that it is critical that the end of the capillary from the fraction collector is positioned to be in contact with the glass wool. Any dead-volume before the glass wool will promote turbulent flow and lead to analyte condensation on the walls of the tubes. However, the capillary termini must not be buried within the glass wool as this can cause blockage that would prevent collection of the analyte. The average trapping efficiency of the glass wool in the STS traps was found to be 95.7 % of the C introduced to the pcGC and the entirety of this C can be combusted directly for graphitization and radiocarbon analysis without any risk of evaporative loss during solvent removal or contamination with residual organic solvent.

Qualitative and quantitative assessment of exogenous carbon contributions

The amount of exogenous carbon introduced to samples isolated by pcGC into the STS traps was quantified by 700 MHz microcryoprobe ^{1}H NMR (detailed results in supporting information Table S2 alongside data for the "U" traps reported by Casanova et al.[16] and Fig. 2B). The mean amount of contaminant C (as a proportion of total C) introduced during trapping into the STS traps was found to be 0.03 ‰. This level of radiocarbon-dead contamination would cause a shift in the determined radiocarbon date of <1 y to older values. This is a lower level of contamination than was determined for compounds isolated in the traditional "U" traps (0.14 ‰; ~1 y shift to older values). Neither of the samples recovered from the "U" traps or the STS traps showed any detectable form of exogenous C other than

column bleed poly(dimethyl siloxanes). The mean amount of column bleed isolated alongside the FAME standards was 28 ng C for the "U" traps and 4 ng C for the STS traps. Neither represent a significant level of contamination, however, it is interesting that less column bleed was trapped using the new STS trap design. This observed difference is unlikely to be due to differences in the condition of the GC column, as these trapping sequences were carried out 1 week apart on the same instrument with the same GC column installed. It could be that the internal walls of the STS trap tube have a higher affinity for trapping poly(dimethyl siloxanes) than the glass wool or that being more volatile, the PDMSs are not retained on the glass wool, but the length of the "U" traps is sufficient to allow their condensation and recovery, although, this has yet to be fully tested experimentally.

Accuracy and precision assessment

The scatter, measured as the standard deviation (SD) of true replicate analyses observed within radiocarbon determinations of replicate isolations and analyses of the same FAME standards was assessed for both trap designs. This gives a measure of the overall precision of the data obtained with each trap design. The radiocarbon determinations were then compared to those performed off-line for the same FAME standard (combusted and graphitized directly without isolation by pcGC) to assess the accuracy of the compound-specific radiocarbon determinations (detailed results in supporting information Table S3). It is clear from Fig. 3 that the scatter observed in the $F^{14}C$ values determined for FAMEs isolated using the traditional "U" traps with solvent recovery (SD=0.0088 and 0.0120 for the $C_{16:0}$ and $C_{18:0}$ FAMEs, respectively) is far higher than the same FAMEs measured off-line (SD=0.0030 and 0.0021 for the $C_{16:0}$ and $C_{18:0}$ FAMEs, respectively). The $F^{14}C$ values of FAMEs isolated using the new STS trap design (SD=0.0041 and 0.0020 for the $C_{16:0}$ and $C_{18:0}$ FAMEs,

309 respectively) demonstrate a much lower degree of scatter than the "U" traps and more closely
310 reflect the accuracy and precision of the $F^{14}C$ values determined without pcGC isolation.

311 Interestingly, the scatter observed in radiocarbon contents of FAMEs isolated from the "U"
312 traps was not solely towards lower $F^{14}C$ values, as would be expected due to differing
313 amounts of radiocarbon 'dead' C from residual solvent. Some replicates demonstrated
314 significantly higher $F^{14}C$ values. The transfer of FAMEs in organic solvents from the "U"
315 traps to tin capsules and the subsequent solvent removal under a stream of N_2 involves much
316 sample handling in the open, and it is possible that additional ('modern') exogenous C could
317 be introduced at this stage[22]. Sources of this more modern exogenous C using this system
318 were not identified in this study, but this further highlights the need for minimal sample
319 handling post-isolation, as enabled by solventless traps. The quick and simple transfer of the
320 glass wool from the STS traps into tin capsules minimizes these sources of contamination.

321 The weighted means of the $F^{14}C$ values determined for the $C_{16:0}$ and $C_{18:0}$ FAME standards
322 and their 1σ uncertainties were determined as 0.9882 ± 0.0015 and 1.0326 ± 0.0014,
323 respectively. The weighted means for the $C_{16:0}$ and $C_{18:0}$ FAMEs from the "U" traps were
324 0.9905 ± 0.0020 and 1.0253 ± 0.0020, respectively, and those from the STS traps were
325 0.9872 ± 0.0015 and 1.0297 ± 0.0014, respectively.

326 A χ^2 test was applied to determine whether replicate radiocarbon analyses of the FAME
327 standards isolated using each trap design demonstrated unacceptably high levels of scatter[23].
328 The χ^2 test compared each replicate with the weighted mean of all replicates and the
329 calculated χ^2 statistic was compared with the critical values for the relevant number of
330 degrees of freedom. The χ^2 test was considered 'passed' if the χ^2 statistic was below the
331 critical value corresponding to the 5% level. The ^{14}C dates obtained for the $C_{16:0}$ and $C_{18:0}$
332 FAMEs isolated using the "U" traps both failed the χ^2 test at the 5 % level (T' = 27.7, T'(5%)

= 9.5, υ = 4 and T' = 28.4, T'(5%) = 9.5, υ = 4, respectively) [23], indicating a far higher level of scatter than would be expected on a purely statistical basis. The $C_{16:0}$ and $C_{18:0}$ FAMEs isolated using the STS traps both passed the χ^2 test at the 5 % level (T' = 6.2, T'(5%) = 14.1, υ = 7 and T' = 1.5, T'(5%) = 14.1, υ = 7, respectively) indicating acceptable levels of sample scatter (and therefore precision). As a further test of the equivalence of the values obtained off-line for the pure FAME standards and those isolated by pcGC using the STS traps, the replicates from both sets of analyses were combined and again subjected to χ^2 tests (both comparing all replicates with the overall weighted mean value and with the weighted mean from the off-line measurements alone) and passed at the 5 % level in each case (T' = 0.2, T'(5%) = 3.8, υ = 1 for the $C_{16:0}$ and T' = 1.9, T'(5%) = 3.8, υ = 1 for the $C_{18:0}$). This not only indicates that the precision of the STS method is excellent, but (in addition to the fact that the weighted means agree to within 2σ) that the dates produced are accurate. The same tests were performed on the replicate measurements from the "U" traps, however, these failed the χ^2 test at the 5 % level in case of the $C_{18:0}$ (T' = 0.8, T'(5%) = 3.8, υ = 1 for the $C_{16:0}$ and T' = 8.9, T'(5%) = 3.8, υ = 1 for the $C_{18:0}$).

It is therefore clear that the use of the new STS trap design avoids the contamination of isolated analytes by residual solvent first raised by Eglinton and co-workers[1] and confirmed unambiguously in this study. The reduced analyte handling between trapping and combustion afforded by the direct transfer of analyte on glass wool to sample capsules minimizes the introduction of exogenous C at this stage, such that the resulting radiocarbon dates are both accurate and precise.

Cross contamination considerations

The possibility for cross contamination between trapping sequences was assessed by GC analysis of the contents of clean trap installed immediately after a typical 40 run trapping sequence with a FAME standard, followed by a solvent only trapping run immediately after installation of clean traps (see supporting information Table S4). The GC analysis showed that residual FAMEs are carried over into the new traps and this is independent of the trap design (Fig. 4A). The amount of FAME transferred into the clean traps ranged from 0.1 to 38.0 µg of C. The variation observed between residual $C_{16:0}$ and $C_{18:0}$ probably relates to the differences in volatility of the analytes and the amount injected. If we consider a typical trapped amount of analyte to be 200 µg of C then the proportion of cross contamination would range from 0.04 % to 13.6 %, which would have significant impact on radiocarbon determinations. This clearly demonstrates a further source of contamination in pcGC and emphasizes the need for cleaning the instrument between trapping sequences.

A simple cleaning method involved the use of a heat gun to effect evaporation of residual condensed compounds from the end of the transfer capillaries connecting the switching valve to the traps. Repeating the analysis described above, but with the use of a heat gun to clean the capillaries following the FAME trapping sequence, confirms that this approach entirely eliminates any FAMEs condensed at the end of the capillaries (Fig. 4B). The method is fast, efficient, and preserves precious sample.

Application of the method to archeological fats

The new trapping method, involving the STS traps and heat gun cleaning, was tested to evaluate accuracy of radiocarbon measurements using archaeological fats of varying age. Bog butters offer a unique material for this study, being found as singly deposited hoards in amounts up to 50 kg (commonly recovered from peat bogs) which have been shown to be

pure fats, largely butter, and are thus composed entirely of fatty acids that can be isolated by pcGC [24, 25]. Critically, due to their purity they present a unique opportunity to rigorously validate the CSRA dating method, as they can be directly radiocarbon dated and used as 'known age' standards for CSRA.

In order to test the homogeneity of the archaeological fats prior to CSRA dating, bulk ^{14}C measurements of 4 bog butters out of 6 selected for CSRA were performed. The triplicate ^{14}C dates of each bog butter were found to be identical within a 2σ error. The fatty acids of the six bog butters that yielded bulk dates of 3,311 ± 26 BP (IB3), 3,069 ± 16 BP (IB1), 2,192 ± 16 BP (IB18), 1,971 ± 16 BP (IB12), 1,153 ± 25 BP (IB6) and 509 ± 16 BP (IB19) were isolated using the STS traps, with the heat gun cleaning between trapping sequences (supporting information Table S5, Fig. S1). These tests were not performed using the U traps as samples isolated in this manner failed to achieve the necessary accuracy and precision[7-9].

Individual ^{14}C dates on the $C_{16:0}$ and $C_{18:0}$ FAs were identical within a 2σ error for each bog butter showing a uniformity of measurements obtained from two different single compounds. Two of the bog butters (IB18 and IB19) were re-sampled, methylated and CSRA performed a second time and no significant differences in the dates were observed, as the χ^2 test at the 5 % level ($T' = 4.5$, $T'(5\%) = 9.5$, $\upsilon = 3$ and $T' = 1.7$, $T'(5\%) = 9.5$, $\upsilon = 4$, respectively) was successfully applied in both cases, highlighting once again excellent reproducibility of the method.

Comparison of the weighted averages of the bulk dates with single ^{14}C determinations on FAMEs showed they were identical within 1 or 2σ error, with one exception, IB18-$C_{16:0}$ (BRAMS-1102.4.1) for which the ^{14}C measurement was just outside the 2σ error of the weighted average. All bulk and CSRA determinations for each of bog butter were subjected jointly to the χ^2 test at the 5 % level, which they all passed successfully (IB1: $T' = 1.9$,

T'(5%) = 7.8, υ = 4; IB3: T' = 4.8, T'(5%) 5.9, υ = 2, IB6: T' = 1.6, T'(5%) = 5.9, υ = 2; IB12: T' = 1.8, T'(5%) = 9.5, υ = 3; IB18: T' = 6.7, T'(5%) = 12.6, υ = 6; IB19: T' = 2.4, T'(5%) = 12.6, υ = 6), indicating statistically identical measurements between bulk and CSRA with an acceptable level of scatter. Thus, there is extremely good agreement between bulk and CSRA dates; this is further emphasized when plotting the CSRA dates against bulk dates (Fig. 5). Over a 3,000 year range the data points can be described by a linear function, $y = 0.9875x + 8.7082$, $R^2 = 0.999$. The slope indicates almost a 1/1 ratio for CSRA/bulk measurements, in addition the line intercepts close to the origin at ~9 years, suggesting no significant offsets exist within the CSRA measurements.

These results demonstrate the possibility for generating radiocarbon dates on single FAs statistically indistinguishable from the bulk fats using the new STS traps combined with cleaning of the capillaries between trapping sequences using the new heat gun method.

Conclusions

The results presented in this paper demonstrate the effectiveness of an entirely new approach to the isolation and handling of individual compounds for high precision ^{14}C determinations. The STS presented completely eliminates the need to use organic solvent for the transfer of isolated compounds to the combustion/graphitization system, thereby overcoming concerns and shortcomings surrounding the previously described trapping system and transfer method. The new STS is extremely simple and can be immediately adopted by any pcGC user after fashioning the new traps as described in this paper (Fig. 1C). The analytes accumulated in the glass wool fitted in the STS trap can be transferred from the traps directly into a tin/aluminum capsule for graphitization without using solvent, which is a major advance for CSRA. The effectiveness of the approach has been assessed through the AMS analysis of a range of

reference and archaeological materials. The validation of the method has also benefited from the application of microcyroprobe ^{1}H NMR technology operating at high field (700 MHz) which allowed the magnitude of contamination by the transfer solvent to be rigorously assessed. The advantages of this new trapping approach include: (i) elimination of organic solvent for handling of isolated compounds, (ii) reduced GC column stationary phase column bleed, (iii) direct transfer of the single compounds from the trap to the tin/foil capsule for graphitization allowing fast recovery of single compounds from the traps, thereby minimizing the introduction of exogenous contaminants prior graphitization, and (v) reproducible and accurate ^{14}C determinations.

A further critical modification has resulted from our identification of a cold spot at the terminus of the deactivated fused silica transfer capillaries connecting the switching value to the borosilicate traps. The cold spot results in condensation of analytes which can contaminate subsequent trapped compounds unless remedial action is taken. This condensate is eliminated very simply through the application of a heat gun between trapping sequences to clean the transfer capillaries; the effectiveness of this was confirmed through the GC analysis of 'blank' trap contents after a trapping sequence. The advantages of the heat gun cleaning method are that it is fast, easy to use and extremely efficient.

Together these modifications constitute significant practical advances in compound-specific radiocarbon analysis of lipids isolated by pcGC. The recognition and elimination of contamination is important to all applications of compound-specific radiocarbon analysis but the minimizing of contamination will be most significant in the area of archeology where the highest precision calendrical dates are demanded.

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References

(1) Eglinton, T. I., Aluwihare, L. I., Bauer, J. E., Druffel, E. R. M. and McNichol, A. P. *Anal. Chem*. **1996,** *68,* 904-912

(2) Currie, L. A., Eglinton, T. I., Benner Jr, B. A. and Pearson, A. *Nucl. Instrum. Meth. B.* **1997,** *123,* 475-486

(3) McNichol, A. P., Ertel, J. R. and Eglinton, T. I. *Radiocarbon*. **2000,** *42,* 219-227

(4) Pearson, A., McNichol, A. P., Benitez-Nelson, B. C., Hayes, J. M. and Eglinton, T. I. *Geochim. Cosmochim. Ac*. **2001,** *65,* 3123-3137

(5) Smittenberg, R. H., Hopmans, E. C., Schouten, S., Hayes, J. M., Eglinton, T. I. and Sinninghe Damsté, J. S. *Paleoceanography*. **2004,** *19,*

(6) Ohkouchi, N. and Eglinton, T. I. *Quat. Geochronol*. **2008,** *3,* 235-243

(7) Stott, A. W., Berstan, R., Evershed, P., Hedges, R. E. M., Ramsey, C. B. and Humm, M. J. *Radiocarbon*. **2001,** *43,* 191-197

(8) Stott, A. W., Berstan, R., Evershed, R. P., Bronk-Ramsey, C., Hedges, R. E. M. and Humm, M. J. *Anal. Chem*. **2003,** *75,* 5037-5045

(9) Berstan, R., Stott, A. W., Minnitt, S., Bronk Ramsey, C., Hedges, R. E. M. and Evershed, R. P. *Antiquity*. **2008,** *82,* 702-713

(10) Santos, G. M., Southon, J. R., Griffin, S., Beaupre, S. R. and Druffel, E. R. M. *Nucl. Instrum. Meth. B*. **2007,** *259,* 293-302

(11) Santos, G. M., Southon, J. R., Drenzek, N. J., Ziolkowski, L. A., Druffel, E. R. M., Xu, X., Zhang, D., Trumbore, S., Eglinton, t. I. and Hunghen, K. A. *Radiocarbon*. **2010,** *52,* 1322-1335

(12) Coppola, A. I., Ziolkowski, L. A. and Druffel, E. R. M. *Radiocarbon*. **2013,** *55,* 1631-1640

(13) Shah, S. R. and Pearson, A. *Radiocarbon*. **2007,** *49,* 69-82

(14) Zencak, Z., Reddy, C. M., Teuten, E. L., Xu, L., McNichol, A. P. and Gustafsson, Ö. *Anal. Chem*. **2007,** *79,* 2042-2049

(15) Ziolkowski, L. A. and Druffel, E. R. M. *Anal. Chem*. **2009,** *81,* 10156-10161

(16) Casanova, E., Knowles, T. D. J., Williams, C., Crump, M. P. and Evershed, R. P. *Anal. Chem*. **2017,** *89,* 7090-7098

(17) Cisneros-Dozal, L. M., Xu, X., Bryant, C., Pearson, E. J. and Dungait, J. A. J. *Radiocarbon*. **2016,** *58,* 445-458

(18)Berstan, R. **2002,** *PhD,*

(19) Smyth, J., Berstan, R., Casanova, E., McCormick, F., Mulhall, I., Sikora, M., Synnott, C. and Evershed, R. P. **Under review,**

(20) Correa-Ascencio, M. and Evershed, R. P. *Anal. Meth*. **2014,** *6,* 1330-1340

(21) Wacker, L., Němec, M. and Bourquin, J. *Nucl. Instrum. Meth. B*. **2010,** *268,* 931-934

(22) Hanke, U. M., Wacker, L., Haghipour, N., Schmidt, M. W. I., Eglinton, T. I. and McIntyre, C. P. *Radiocarbon*. **2017,** *59,* 1103-1116

(23) Ward, G. K. and Wilson, S. R. *Archaeometry*. **1978,** *20,* 19-31

(24) Earwood, C. *J. Irish Arch*. **1997,** *8,* 25-42

(25) Berstan, R., Dudd, S. N., Copley, M. S., Morgan, E. D., Quye, A. and Evershed, R. P. *Analyst*. **2004,** *129,* 270-275

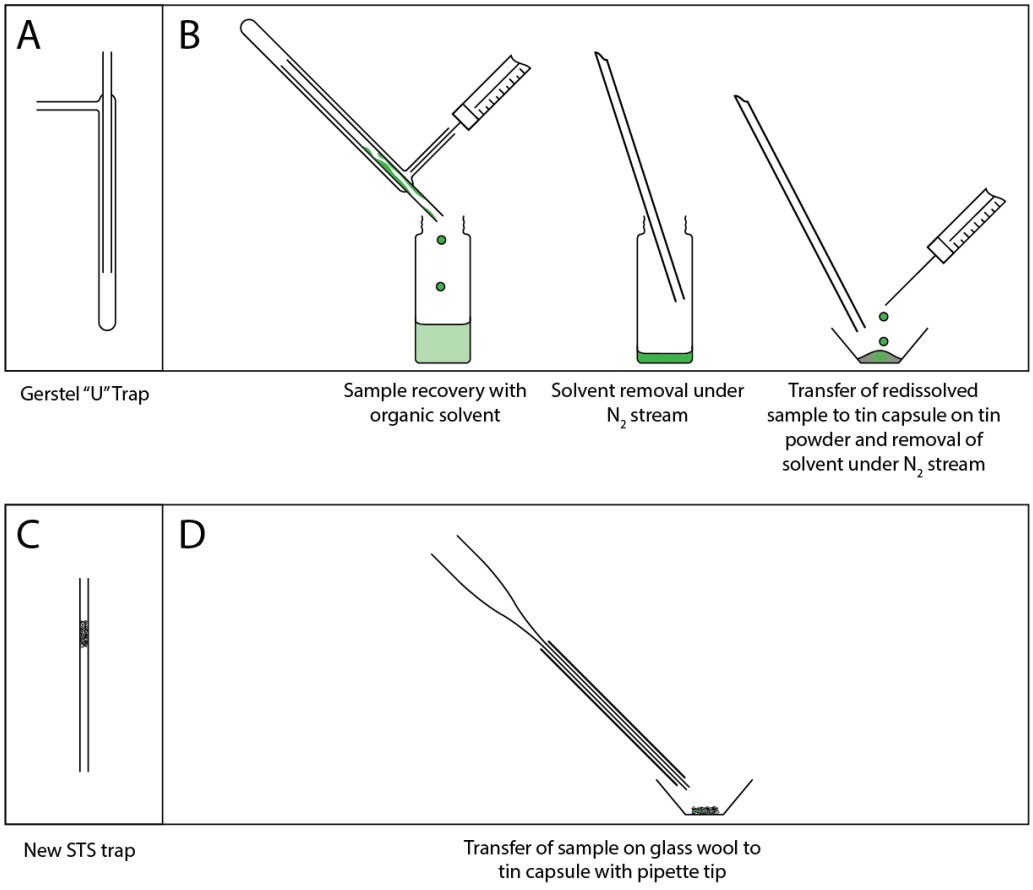


Figure 1: (A) Gerstel "U" traps. (B) Sample recovery method for "U" traps. (C) New solventless trapping system (STS) traps. (D) Sample recovery method for STS traps.

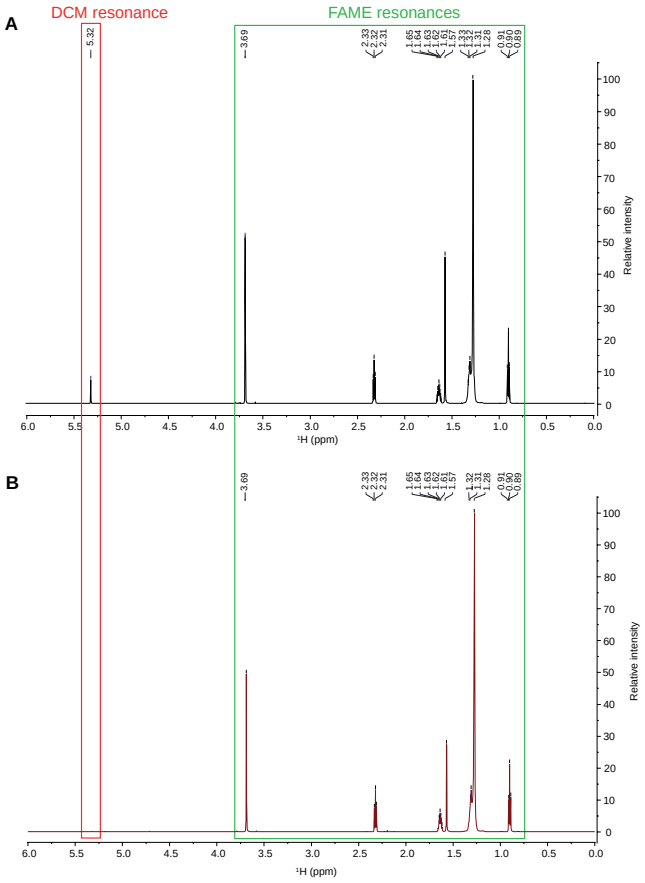


Figure 2: (A) Partial ^{1}H NMR spectrum of $C_{16:0}$ isolated in "U" trap, recovered with DCM and blown down to dryness prior to NMR analysis. (B) Partial ^{1}H NMR spectrum of $C_{16:0}$ isolated in STS trap without using solvent for the recovery. The resonances between 0.89 ppm and 3.69 ppm derived from the $C_{16:0}$ FAME[15] and the resonance at 5.32 ppm corresponds to DCM.

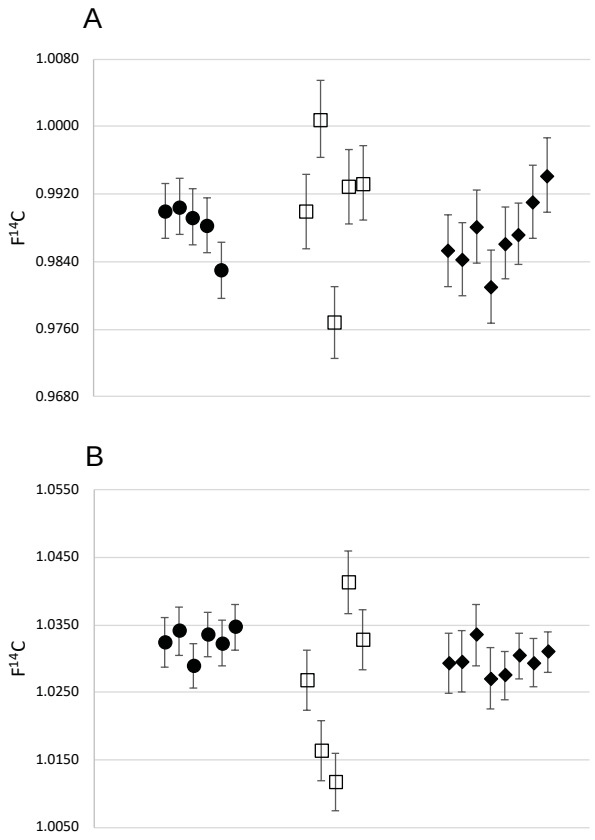
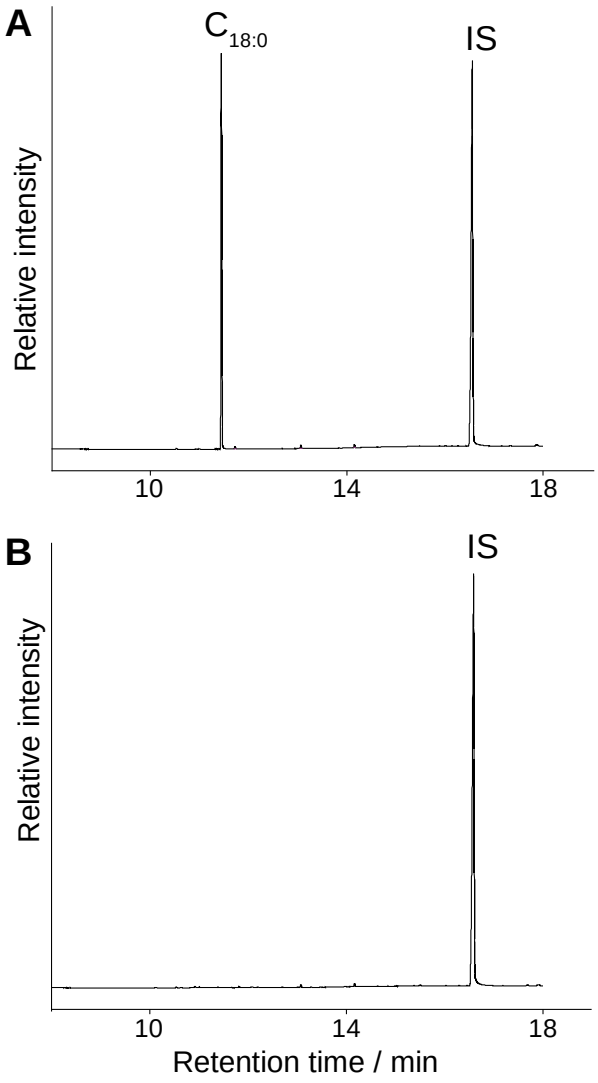


Figure 3: $F^{14}C$ values of (A) the $C_{16:0}$ and (B) $C_{18:0}$ FAME standards. Black dots represent off-line measurements, white squares represent compounds isolated in the "U" traps and black diamonds represent compounds isolated in the STS traps. The error bars correspond the 1σ analytical uncertainty.



522

523 Figure 4: Partial gas chromatograms of the trap contents (T2($C_{18:0}$)) of pure solvent injection
524 after a trapping sequence of the FAME standard solution. (A) No cleaning of the capillaries
525 prior to solvent injection and (B) cleaning of the capillary with a heat gun prior to solvent
526 injection. IS is the internal standard.

527

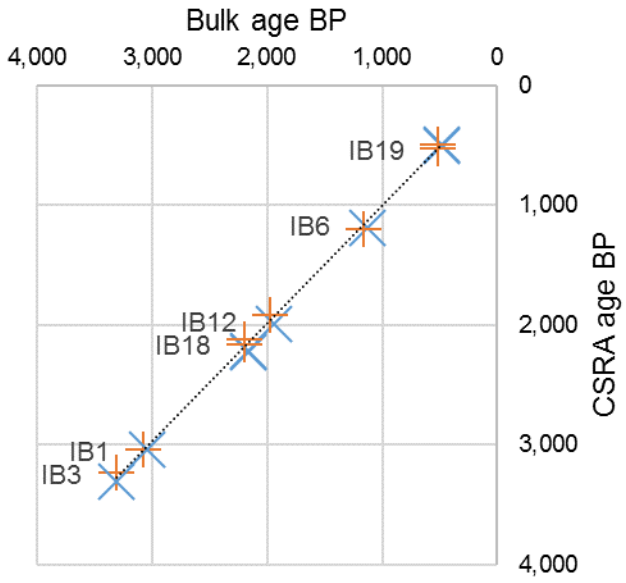
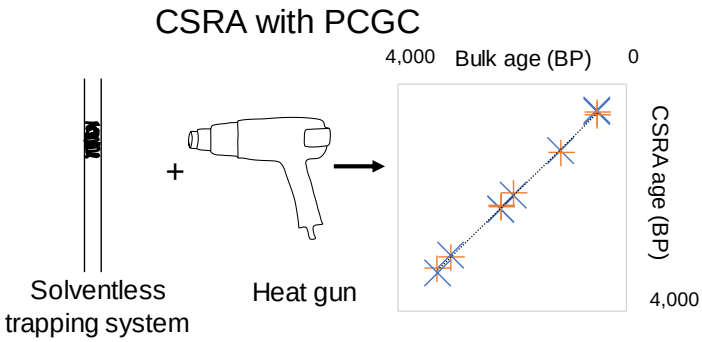


Figure 5: CSRA measurements (in years BP) plotted against the weighted average of bulk measurements for 6 bog butters of age ranging between 3,000-500 BP. The $C_{16:0}$ FAs dates are represented by "x" and "$C_{18:0}$ FAs by "+". Dashed line corresponds to the linear trendline modelled for the data points ($y = 0.9875x + 8.7082$, $R^2 = 0.999$).

For TOC only


CSRA with PCGC
4,000
Bulk age (BP)
0
CSRA age (BP)
4,000
Solventless trapping system
Heat gun