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1 **Practical considerations in high-precision compound-specific radiocarbon analyses:**

2 **eliminating the effects of solvent and sample cross-contamination on accuracy and**

3 **precision**

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15

16 **Abstract**

17 Preparative capillary gas chromatography (pcGC) is widely used for the isolation of single

18 compounds for radiocarbon determinations. While being effective at isolating compounds,

19 there are still genuine concerns relating to contamination associated with the isolation

20 procedure, such as incomplete removal of solvent used to recover isolated samples from the

21 traps and cross-contamination, which can lead to erroneous ^{14}C determinations. Herein we

22 describe new approaches to identifying and removing these two sources of contamination.

23 First, we replaced the common "U" trap design, which requires recovery of compounds using

24   organic solvent, with a novel solventless trapping system (STS), consisting of a simple glass

25   tube fitted with a glass wool plug, allowing the condensation of isolated compound in the

26   wool and their solventless recovery by pushing the glass wool directly into a foil capsule for

27   graphitization. With the STS trap, an average of 95.7 % of the isolated compound was

28   recovered and contamination from column bleed was reduced. In addition, comparison of ^{14}C

29   determinations of fatty acid methyl ester (FAME) standards determined offline to those

30   isolated by pcGC in STS traps showed excellent reproducibility and accuracy compared to

31   those isolated using the traditional "U" traps. Second, "cold-spots" were identified on the

32   instrument, i.e. the termini of capillaries in the preparative unit, which can be cleaned of

33   compounds condensed from earlier runs using a heat gun. Our new procedure, incorporating

34   these two modifications, was tested on archaeological fat hoards, producing ^{14}C dates on

35   isolated $C_{16:0}$ and $C_{18:0}$ fatty acids statistically consistent with the bulk dates of the

36   archaeological material.

37

38   **Introduction**

39   Compound-specific radiocarbon analysis (CSRA) has proven to be a powerful tool in

40   understanding C dynamics in the earth system at a mechanistic/process level by allowing the

41   fluxes and turnover rates of individual compounds or compound classes with well-defined

42   sources to be determined. The use of preparative capillary gas chromatography (pcGC) for

43   the isolation of pure compounds for CSRA has been used widely in the environmental

44   sciences since its inception by Eglinton and co-workers[1] wherein the authors demonstrated

45   the application of the technique to radiocarbon determinations of *n*-alkanes and *n*-fatty acids.

46   The technique has since been extended to the analysis of a range of various organic

47   compounds in a diversity of matrices[2-6].

48   An important potential application of this approach is the radiocarbon dating of archeological

49   organic residues, particularly the $C_{16:0}$ and $C_{18:0}$ fatty acids derived from animal fats which

50   occur widely adsorbed within the clay matrix of archaeological potsherds. Although the

51   results of initial studies[7-9] were promising, it was evident that the accuracy and precision of

52   these determinations did not meet the high standards demanded by archaeological dating,

53   making this perhaps the most challenging application of the technique. Any exogenous C

54   added to the samples (especially on small sample sizes) isolated from the archaeological

55   matrix would reduce the accuracy of ^{14}C measurements and require correction[10, 11].

56   Furthermore, it is critical for many archaeological applications that small chronological

57   differences between samples (and therefore events) can be observed after calibration. The

58   accuracy and precision that is therefore required for archaeological samples exceeds those

59   often deemed reasonable for other CSRA applications.

60   Assuming sufficient analyte C is available, the major factors which can limit the achievable

61   levels of accuracy and precision during compound-specific radiocarbon dating are: i)

62   introduction of exogenous C during isolation by pcGC, oxidation and reduction to graphite;

63   and ii) cross contamination or sample "carryover" between samples within the pcGC system

64   (i.e. GC or preparative fraction collector (PFC)). The samples and standards prepared in this

65   study were not considered to be 'small', however, for applications where only small samples

66   can be isolated and analyzed (ca. 10-100 μg C), the assessment of blank contributions and

67   correcting for their effects is critical and much research has been dedicated to such studies[10-

68   13].

69   The matter of contamination of analytes with exogenous C incorporated during isolation of

70   compounds by pcGC, whether from column "bleed" and/or due to residual solvent in isolated

71   compounds after drying, has always been a matter of concern for users of the technique [1, 11,

72   14, 15]. In their original validation of the method, Eglinton et al.[1] used capillary columns coated

73   with thin films of stationary phase to limit the effects of column "bleed" arising through

74   thermal degradation, concluding that "There is minimal ^{14}C background contamination (<<5

75   µg of C) introduced by the pcGC system or by the GC column". Recently, we demonstrated

76   for the first time that although present in analytes isolated by pcGC, cyclic poly(dimethyl

77   siloxanes) resulting from the degradation of the stationary phase of the GC column, were not

78   present in sufficient quantities to affect high precision ^{14}C determinations[16].

79   The potential for organic solvents, used to manipulate analytes post-trapping, to persist after

80   drying of compounds appears not to have been systematically investigated. The commercially

81   available glass traps used with the Gerstel PFC consist of a coaxial glass tube fitted with a

82   side-arm (Fig. 1A) whereby the column eluent flows down the interior channel, before

83   flowing up the exterior and leaving the trap via a side-arm. It is this outward flow which is

84   switched on and off by the PFC system to direct the column eluent to different traps. The

85   isolated compounds are generally trapped when they condense in the initial few mm of the

86   trap. They are subsequently recovered by back-flushing the trap with an organic solvent

87   followed by removal of the solvent from the resulting solution by blowing down under a

88   gentle stream of N_2 (Fig.1B). Since the compounds isolated by pcGC are generally lipophilic,

89   their affinity for organic solvents could result in the incomplete removal of the organic

90   solvents used to remove them from the traps. The low quantities of analyte trapped, combined

91   with their often relatively high volatilities means that it is undesirable to blow analytes down

92   too strongly or for too long, as this could result in evaporative losses. Considering that any

93   exogenous C present at the permil level would have a significant effect on the determined

94   radiocarbon date of an isolated compound, it is conceivable that this could represent a

95   significant source of exogenous C in such samples. Indeed, although they were unable to

96   identify or quantify any residual solvent using high-resolution GCMS or shifts in δ^{13}C values,

97   in 1996 Eglinton et al.[1] noted that, "Incomplete removal of solvent prior to combustion is the

98   major potential source of carbon contamination" in compounds isolated by pcGC. Commonly

99   adopted characterizations of, and corrections for, the effect of this contamination are

100   performed using the deviation of the determined $F^{14}C$ values for standards and blank

101   materials covering a range of sample sizes[10, 11, 15]. An obvious solution to the effects of

102   incomplete solvent removal would be to recover isolated compounds without the use of

103   organic solvents.

104   A further challenge recognized in the radiocarbon determination of organic compounds

105   isolated by pcGC is cross-contamination between trapping sequences[15, 12, 17]. Strategies for

106   avoiding cross-contamination involve "washing" the entire system by performing repeat

107   injections (10x) of aliquots of the new sample, discarding the resulting isolates and replacing

108   the traps with clean[15, 12, 17]. This practice highly is undesirable, as it constitutes loss of

109   precious analyte especially given that isolating enough C is one of the major challenges in

110   CSRA. Furthermore, the efficacy of this practice has not, as far as we know, been rigorously

111   tested. It is most likely that any cross-contamination occurs as a result of compounds from

112   earlier trapping sequences becoming condensed at 'cold spots' in the pcGC system but are re-

113   mobilized, contaminating the subsequently isolated compound. The most likely location for

114   this to occur is where the fused silica capillaries protrude from the heated sections of the PFC

115   unit and enter the unheated glass traps. We propose that any residual analyte adhering to the

116   capillaries at these locations could be removed with the application of heat.

117   Herein, we report a new trap design and the results of experiments conducted to: i) quantify

118   residual transfer solvent persisting in analytes, trapped using the traditional Gerstel "U" traps,

119   after blowing to dryness under a stream of N_2, ii) compare the sample trapping efficiency,

120   the mass of exogenous carbon introduced, and both the accuracy of ^{14}C determinations (by

121   comparison with off-line preparation) and the precision (scatter of ^{14}C dates) observed in

122   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

Materials and Methods

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 ^1H NMR as described

165   by Casanova et al.[16].

166

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

168   FAMEs standards isolated using both trap designs were quantified by GC-FID (flame

169   ionization detector) after either flushing traps into glass vials with 1 mL of hexane ("U"

170   traps) or by transferring the glass wool into glass vials by pushing it out using the tip of a pre-

171   combusted glass Pasteur pipette and dissolving the trapped FAMEs in hexane (STS traps).

172   This extract was split for both GC-FID analysis, to determine trapping efficiency, and the

173   quantification of exogenous C by NMR. After removal of glass wool, any remaining FAMES

174   on the inside of the STS traps were extracted with hexane to assess the partitioning of trapped

175   compounds between the glass wool and the walls of the glass tubes. Compounds were

176   quantified by GC-FID using the internal standard method.

177

178   **Quantification of exogenous carbon**

179   FAME standards isolated by pcGC using both trap designs were extracted and ¼ of the

180   extract transferred to 1.7 mm NMR tubes in deuterated chloroform and analyzed using 700

181   MHz spectrometer equipped with microcryoprobe for the quantification of poly(dimethyl

182   siloxanes), deriving from 'column-bleed', and screening for residual solvent and other

183   sources of exogenous C as described previously [16].

184

185   **Accuracy and precision of radiocarbon dates**

186   Compounds isolated by pcGC (~200 µg C) were transferred to tin capsules by either (i) using

187   the 'Russian doll' technique outlined by Stott et al.[8] in the case of the traditional "U" traps; or

188   (ii) direct transfer of glass wool to a tin capsule in the case of the new STS traps. Samples

189   (including the pure FAME standards) were combusted using a Vario Isotope Select elemental

190   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 ^1H 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

238   need for using a solventless system for the recovery of compounds isolated by pcGC for

239   radiocarbon dating for archeological applications.

240

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

242   **Description of STS-trap design**

243   The trap design for a solventless recovery (STS trap) tested herein consists of a borosilicate

244   glass capillary (3 mm OD, 1 mm ID, 70 mm in length) containing a 10 mm glass wool plug

245   positioned 15 mm from the top of the trap (Fig. 1C). The capillary tubes are connected to the

246   PFC via PTFE ferrules in the same manner as the "U" traps and the silicone tube connecting

247   the trap to the valve cluster in the PFC is attached to the bottom of the STS traps. The analyte

248   is condensed onto the glass wool, which can be physically removed from the trap by pushing,

249   with the tip of a pre-combusted glass Pasteur pipette, directly into a tin/foil capsule for

250   combustion in an elemental analyzer or into a glass tube for offline combustion (Fig. 1D).

251

252   **Determination of trapping efficiency**

253   The percentages of C lost to waste (trap T0), successfully trapped and recovered in the "U"

254   and STS traps, and in the case of the STS traps, lost to the walls of the traps, were determined

255   by GC-FID (detailed results in supporting information Table S1). The proportion of C from

256   the FAME which was collected in the 'waste' trap, T0, was found to be 1.3 % in the case of

257   the "U" traps, and 1.2 % in the case of the STS traps. Any C transferred to the waste trap is

258   likely to be a consequence of the switching of the traps during the tail of the chromatographic

259   peaks and should therefore be independent of the trap design; our data support this as the

260   amount of FAMEs lost in the waste is identical for both designs.

261   Using the STS trap, a potential source of analyte loss would be due to condensation on the

262   internal walls of the glass tube. Determination of the proportion of C lost on the sides of the

263   tubes in the STS traps was shown to be 3.2 ± 4.4 % (1σ). Losses of up to 12.2 % were

264   observed for the two most extreme cases, in traps where the PFC capillary was not in contact

265   with the glass wool. It can therefore be concluded that it is critical that the end of the

266   capillary from the fraction collector is positioned to be in contact with the glass wool. Any

267   dead-volume before the glass wool will promote turbulent flow and lead to analyte

268   condensation on the walls of the tubes. However, the capillary termini must not be buried

269   within the glass wool as this can cause blockage that would prevent collection of the analyte.

270   The average trapping efficiency of the glass wool in the STS traps was found to be 95.7 % of

271   the C introduced to the pcGC and the entirety of this C can be combusted directly for

272   graphitization and radiocarbon analysis without any risk of evaporative loss during solvent

273   removal or contamination with residual organic solvent.

274

275   **Qualitative and quantitative assessment of exogenous carbon contributions**

276   The amount of exogenous carbon introduced to samples isolated by pcGC into the STS traps

277   was quantified by 700 MHz microcryoprobe ^{1}H NMR (detailed results in supporting

278   information Table S2 alongside data for the "U" traps reported by Casanova et al.[16] and Fig.

279   2B). The mean amount of contaminant C (as a proportion of total C) introduced during

280   trapping into the STS traps was found to be 0.03 ‰. This level of radiocarbon-dead

281   contamination would cause a shift in the determined radiocarbon date of <1 y to older values.

282   This is a lower level of contamination than was determined for compounds isolated in the

283   traditional "U" traps (0.14 ‰; ~1 y shift to older values). Neither of the samples recovered

284   from the "U" traps or the STS traps showed any detectable form of exogenous C other than

285   column bleed poly(dimethyl siloxanes). The mean amount of column bleed isolated alongside

286   the FAME standards was 28 ng C for the "U" traps and 4 ng C for the STS traps. Neither

287   represent a significant level of contamination, however, it is interesting that less column

288   bleed was trapped using the new STS trap design. This observed difference is unlikely to be

289   due to differences in the condition of the GC column, as these trapping sequences were

290   carried out 1 week apart on the same instrument with the same GC column installed. It could

291   be that the internal walls of the STS trap tube have a higher affinity for trapping

292   poly(dimethyl siloxanes) than the glass wool or that being more volatile, the PDMSs are not

293   retained on the glass wool, but the length of the "U" traps is sufficient to allow their

294   condensation and recovery, although, this has yet to be fully tested experimentally.

295

296   **Accuracy and precision assessment**

297   The scatter, measured as the standard deviation (SD) of true replicate analyses observed

298   within radiocarbon determinations of replicate isolations and analyses of the same FAME

299   standards was assessed for both trap designs. This gives a measure of the overall precision of

300   the data obtained with each trap design. The radiocarbon determinations were then compared

301   to those performed off-line for the same FAME standard (combusted and graphitized directly

302   without isolation by pcGC) to assess the accuracy of the compound-specific radiocarbon

303   determinations (detailed results in supporting information Table S3). It is clear from Fig. 3

304   that the scatter observed in the $F^{14}C$ values determined for FAMEs isolated using the

305   traditional "U" traps with solvent recovery (SD=0.0088 and 0.0120 for the $C_{16:0}$ and $C_{18:0}$

306   FAMEs, respectively) is far higher than the same FAMEs measured off-line (SD=0.0030 and

307   0.0021 for the $C_{16:0}$ and $C_{18:0}$ FAMEs, respectively). The $F^{14}C$ values of FAMEs isolated

308   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%)

333 = 9.5, $\upsilon = 4$ and T' = 28.4, T'(5%) = 9.5, $\upsilon = 4$, respectively) [23], indicating a far higher level

334 of scatter than would be expected on a purely statistical basis. The $C_{16:0}$ and $C_{18:0}$ FAMEs

335 isolated using the STS traps both passed the χ^2 test at the 5 % level (T' = 6.2, T'(5%) = 14.1,

336 $\upsilon = 7$ and T' = 1.5, T'(5%) = 14.1, $\upsilon = 7$, respectively) indicating acceptable levels of

337 sample scatter (and therefore precision). As a further test of the equivalence of the values

338 obtained off-line for the pure FAME standards and those isolated by pcGC using the STS

339 traps, the replicates from both sets of analyses were combined and again subjected to χ^2 tests

340 (both comparing all replicates with the overall weighted mean value and with the weighted

341 mean from the off-line measurements alone) and passed at the 5 % level in each case (T' =

342 0.2, T'(5%) = 3.8, $\upsilon = 1$ for the $C_{16:0}$ and T' = 1.9, T'(5%) = 3.8, $\upsilon = 1$ for the $C_{18:0}$). This

343 not only indicates that the precision of the STS method is excellent, but (in addition to the

344 fact that the weighted means agree to within 2σ) that the dates produced are accurate. The

345 same tests were performed on the replicate measurements from the "U" traps, however, these

346 failed the χ^2 test at the 5 % level in case of the $C_{18:0}$ (T' = 0.8, T'(5%) = 3.8, $\upsilon = 1$ for the

347 $C_{16:0}$ and T' = 8.9, T'(5%) = 3.8, $\upsilon = 1$ for the $C_{18:0}$).

348 It is therefore clear that the use of the new STS trap design avoids the contamination of

349 isolated analytes by residual solvent first raised by Eglinton and co-workers[1] and confirmed

350 unambiguously in this study. The reduced analyte handling between trapping and combustion

351 afforded by the direct transfer of analyte on glass wool to sample capsules minimizes the

352 introduction of exogenous C at this stage, such that the resulting radiocarbon dates are both

353 accurate and precise.

354

355 **Cross contamination considerations**

356   The possibility for cross contamination between trapping sequences was assessed by GC

357   analysis of the contents of clean trap installed immediately after a typical 40 run trapping

358   sequence with a FAME standard, followed by a solvent only trapping run immediately after

359   installation of clean traps (see supporting information Table S4). The GC analysis showed

360   that residual FAMEs are carried over into the new traps and this is independent of the trap

361   design (Fig. 4A). The amount of FAME transferred into the clean traps ranged from 0.1 to

362   38.0 µg of C. The variation observed between residual $C_{16:0}$ and $C_{18:0}$ probably relates to the

363   differences in volatility of the analytes and the amount injected. If we consider a typical

364   trapped amount of analyte to be 200 µg of C then the proportion of cross contamination

365   would range from 0.04 % to 13.6 %, which would have significant impact on radiocarbon

366   determinations. This clearly demonstrates a further source of contamination in pcGC and

367   emphasizes the need for cleaning the instrument between trapping sequences.

368   A simple cleaning method involved the use of a heat gun to effect evaporation of residual

369   condensed compounds from the end of the transfer capillaries connecting the switching valve

370   to the traps. Repeating the analysis described above, but with the use of a heat gun to clean

371   the capillaries following the FAME trapping sequence, confirms that this approach entirely

372   eliminates any FAMEs condensed at the end of the capillaries (Fig. 4B). The method is fast,

373   efficient, and preserves precious sample.

374

375   **Application of the method to archeological fats**

376   The new trapping method, involving the STS traps and heat gun cleaning, was tested to

377   evaluate accuracy of radiocarbon measurements using archaeological fats of varying age.

378   Bog butters offer a unique material for this study, being found as singly deposited hoards in

379   amounts up to 50 kg (commonly recovered from peat bogs) which have been shown to be

380 pure fats, largely butter, and are thus composed entirely of fatty acids that can be isolated by

381 pcGC [24, 25]. Critically, due to their purity they present a unique opportunity to rigorously

382 validate the CSRA dating method, as they can be directly radiocarbon dated and used as

383 'known age' standards for CSRA.

384 In order to test the homogeneity of the archaeological fats prior to CSRA dating, bulk ^{14}C

385 measurements of 4 bog butters out of 6 selected for CSRA were performed. The triplicate ^{14}C

386 dates of each bog butter were found to be identical within a 2σ error. The fatty acids of the

387 six bog butters that yielded bulk dates of 3,311 ± 26 BP (IB3), 3,069 ± 16 BP (IB1), 2,192 ±

388 16 BP (IB18), 1,971 ± 16 BP (IB12), 1,153 ± 25 BP (IB6) and 509 ± 16 BP (IB19) were

389 isolated using the STS traps, with the heat gun cleaning between trapping sequences

390 (supporting information Table S5, Fig. S1). These tests were not performed using the U traps

391 as samples isolated in this manner failed to achieve the necessary accuracy and precision[7-9].

392 Individual ^{14}C dates on the $C_{16:0}$ and $C_{18:0}$ FAs were identical within a 2σ error for each bog

393 butter showing a uniformity of measurements obtained from two different single compounds.

394 Two of the bog butters (IB18 and IB19) were re-sampled, methylated and CSRA performed a

395 second time and no significant differences in the dates were observed, as the χ^2 test at the 5 %

396 level (T' = 4.5, T'(5%) = 9.5, υ = 3 and T' = 1.7, T'(5%) = 9.5, υ = 4, respectively) was

397 successfully applied in both cases, highlighting once again excellent reproducibility of the

398 method.

399 Comparison of the weighted averages of the bulk dates with single ^{14}C determinations on

400 FAMEs showed they were identical within 1 or 2σ error, with one exception, IB18-$C_{16:0}$

401 (BRAMS-1102.4.1) for which the ^{14}C measurement was just outside the 2σ error of the

402 weighted average. All bulk and CSRA determinations for each of bog butter were subjected

403 jointly to the χ^2 test at the 5 % level, which they all passed successfully (IB1: T' = 1.9,

404   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;

405   IB12: T' = 1.8, T'(5%) = 9.5, υ = 3; IB18: T' = 6.7, T'(5%) = 12.6, υ = 6; IB19: T' = 2.4,

406   T'(5%) = 12.6, υ = 6), indicating statistically identical measurements between bulk and

407   CSRA with an acceptable level of scatter. Thus, there is extremely good agreement between

408   bulk and CSRA dates; this is further emphasized when plotting the CSRA dates against bulk

409   dates (Fig. 5). Over a 3,000 year range the data points can be described by a linear function, y

410   = 0.9875x + 8.7082, R² = 0.999. The slope indicates almost a 1/1 ratio for CSRA/bulk

411   measurements, in addition the line intercepts close to the origin at ~9 years, suggesting no

412   significant offsets exist within the CSRA measurements.

413   These results demonstrate the possibility for generating radiocarbon dates on single FAs

414   statistically indistinguishable from the bulk fats using the new STS traps combined with

415   cleaning of the capillaries between trapping sequences using the new heat gun method.

416

417   **Conclusions**

418   The results presented in this paper demonstrate the effectiveness of an entirely new approach

419   to the isolation and handling of individual compounds for high precision ^{14}C determinations.

420   The STS presented completely eliminates the need to use organic solvent for the transfer of

421   isolated compounds to the combustion/graphitization system, thereby overcoming concerns

422   and shortcomings surrounding the previously described trapping system and transfer method.

423   The new STS is extremely simple and can be immediately adopted by any pcGC user after

424   fashioning the new traps as described in this paper (Fig. 1C). The analytes accumulated in the

425   glass wool fitted in the STS trap can be transferred from the traps directly into a tin/aluminum

426   capsule for graphitization without using solvent, which is a major advance for CSRA. The

427   effectiveness of the approach has been assessed through the AMS analysis of a range of

428 reference and archaeological materials. The validation of the method has also benefited from

429 the application of microcyroprobe ^{1}H NMR technology operating at high field (700 MHz)

430 which allowed the magnitude of contamination by the transfer solvent to be rigorously

431 assessed. The advantages of this new trapping approach include: (i) elimination of organic

432 solvent for handling of isolated compounds, (ii) reduced GC column stationary phase column

433 bleed, (iii) direct transfer of the single compounds from the trap to the tin/foil capsule for

434 graphitization allowing fast recovery of single compounds from the traps, thereby minimizing

435 the introduction of exogenous contaminants prior graphitization, and (v) reproducible and

436 accurate ^{14}C determinations.

437 A further critical modification has resulted from our identification of a cold spot at the

438 terminus of the deactivated fused silica transfer capillaries connecting the switching value to

439 the borosilicate traps. The cold spot results in condensation of analytes which can

440 contaminate subsequent trapped compounds unless remedial action is taken. This condensate

441 is eliminated very simply through the application of a heat gun between trapping sequences to

442 clean the transfer capillaries; the effectiveness of this was confirmed through the GC analysis

443 of 'blank' trap contents after a trapping sequence. The advantages of the heat gun cleaning

444 method are that it is fast, easy to use and extremely efficient.

445 Together these modifications constitute significant practical advances in compound-specific

446 radiocarbon analysis of lipids isolated by pcGC. The recognition and elimination of

447 contamination is important to all applications of compound-specific radiocarbon analysis but

448 the minimizing of contamination will be most significant in the area of archeology where the

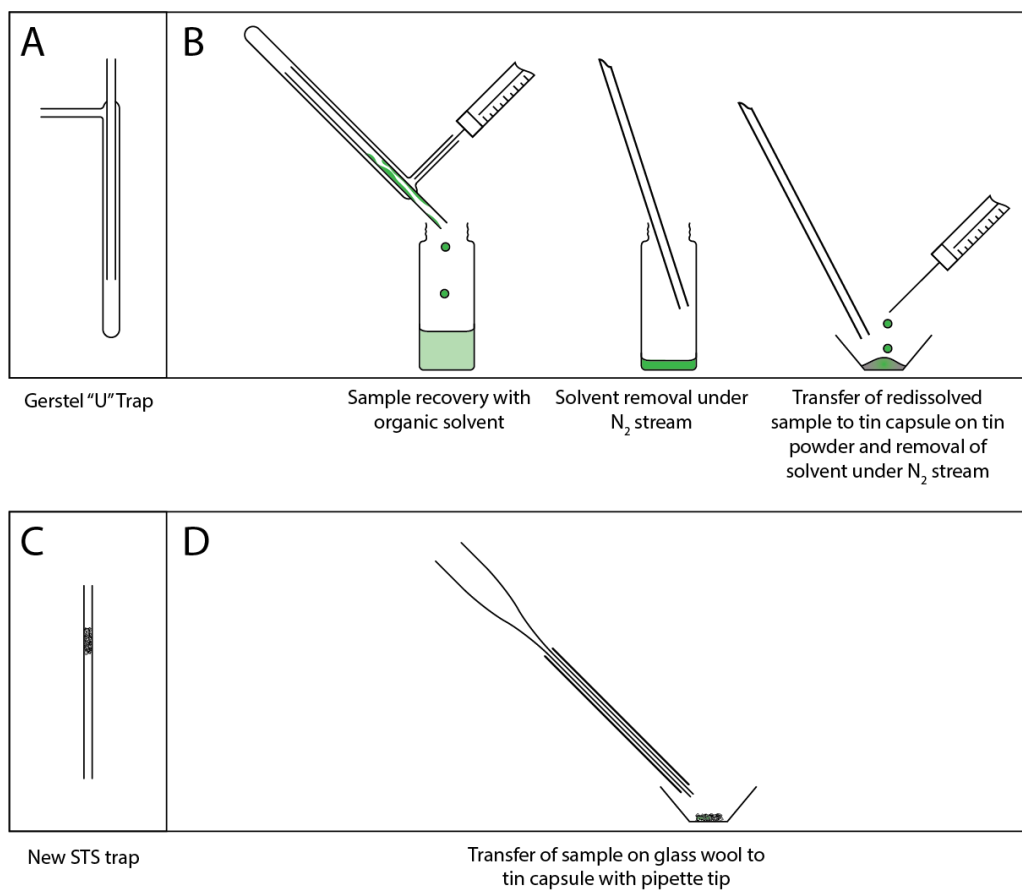449 highest precision calendrical dates are demanded.

450

451 **Acknowledgements**

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A Gerstel "U" Trap

B Sample recovery with organic solvent   Solvent removal under N_2 stream   Transfer of redissolved sample to tin capsule on tin powder and removal of solvent under N_2 stream

C New STS trap

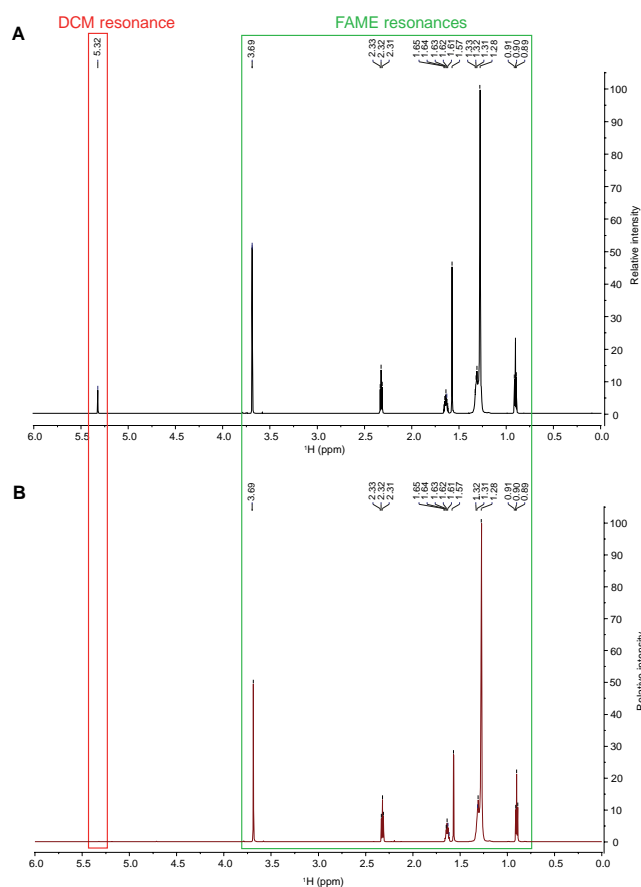**D** Transfer of sample on glass wool to tin capsule with pipette tip

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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.
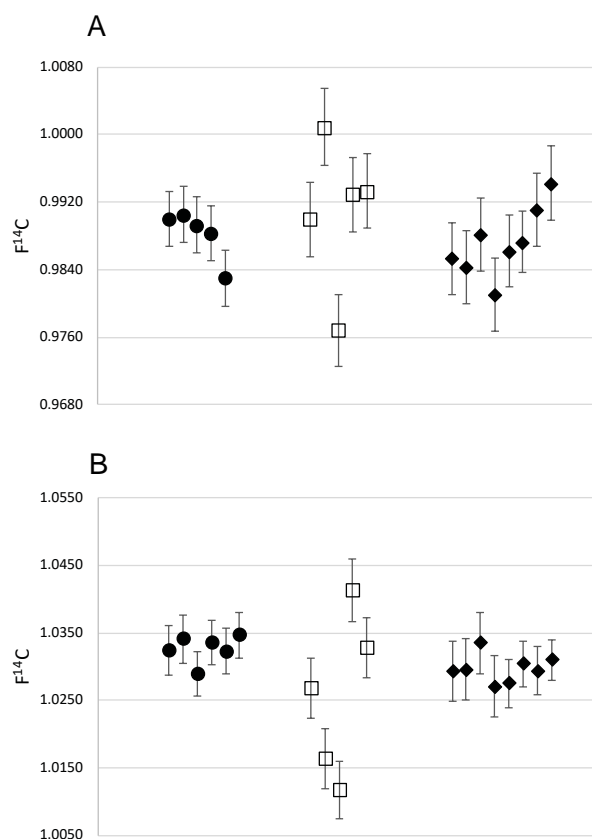
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Figure 2: (A) Partial ^{1}H NMR spectrum of $C_{16:0}$ isolated in "U" trap, recovered with DCM and blown down to dryness prior to NMR analysis. (B) Partial ^{1}H NMR spectrum of $C_{16:0}$ isolated in STS trap without using solvent for the recovery. 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.
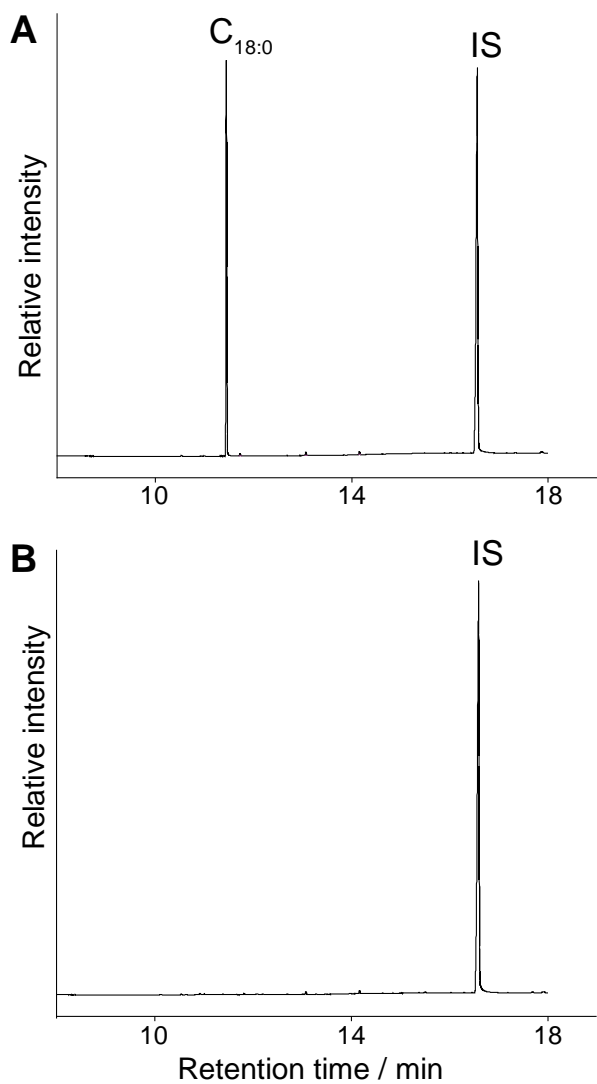
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516

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.

521

A

$C_{18:0}$ IS

Relative intensity

10   14   18

B

IS

Relative intensity

10   14   18

Retention time / min

522

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

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528
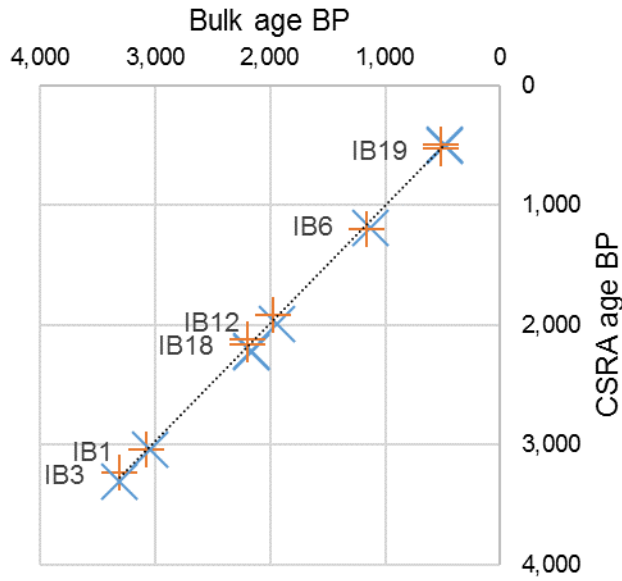
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530 Figure 5: CSRA measurements (in years BP) plotted against the weighted average of bulk
531 measurements for 6 bog butters of age ranging between 3,000-500 BP. The $C_{16:0}$ FAs dates
532 are represented by "x" and "$C_{18:0}$ FAs by "+". Dashed line corresponds to the linear trendline
533 modelled for the data points (y = 0.9875x + 8.7082, R² = 0.999).

534

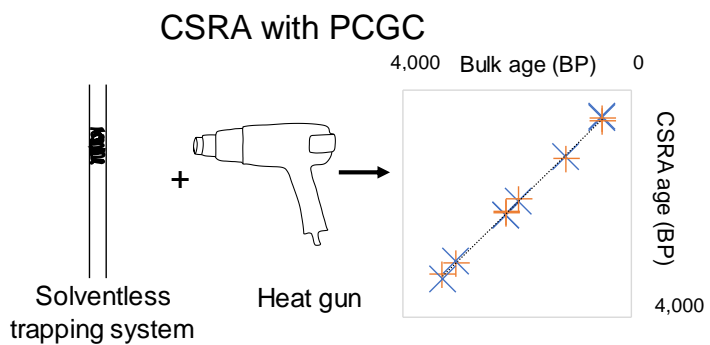535

536

For TOC only

CSRA with PCGC