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^{14}C BLANK ASSESSMENT IN SMALL-SCALE COMPOUND-SPECIFIC RADIOCARBON ANALYSIS OF LIPID BIOMARKERS AND LIGNIN PHENOLS

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ABSTRACT. Compound-specific radiocarbon (^{14}C) dating often requires working with small samples of < 100 µg carbon (µgC). This makes the radiocarbon dates of biomarker compounds very sensitive to biases caused by extraneous carbon of unknown composition, a procedural blank, which is introduced to the samples during the steps necessary to prepare a sample for radiocarbon analysis by accelerator mass spectrometry (i.e., isolating single compounds from a heterogeneous mixture, combustion, gas purification and graphitization). Reporting accurate radiocarbon dates thus requires a correction for the procedural blank. We present our approach to assess the fraction modern carbon ($F^{14}C$) and the mass of the procedural blanks introduced during the preparation procedures of lipid biomarkers (i.e. *n*-alkanoic acids) and lignin phenols. We isolated differently sized aliquots (6–151 µgC) of *n*-alkanoic acids and lignin phenols obtained from standard materials with known $F^{14}C$ values. Each compound class was extracted from two standard materials (one fossil, one modern) and purified using the same procedures as for natural samples of unknown $F^{14}C$. There is an inverse linear relationship between the measured $F^{14}C$ values of the processed aliquots and their mass, which suggests constant contamination during processing of individual samples. We use Bayesian methods to fit linear regression lines between $F^{14}C$ and 1/mass for the fossil and modern standards. The intersection points of these lines are used to infer $F^{14}C_{blank}$ and m_{blank} and their associated uncertainties. We estimate 4.88 ± 0.69 µgC of procedural blank with $F^{14}C$ of 0.714 ± 0.077 for *n*-alkanoic acids, and 0.90 ± 0.23 µgC of procedural blank with $F^{14}C$ of 0.813 ± 0.155 for lignin phenols. These $F^{14}C_{blank}$ and m_{blank} can be used to correct AMS results of lipid and lignin samples by isotopic mass balance. This method may serve as a standardized procedure for blank assessment in small-scale radiocarbon analysis.

KEYWORDS: blank assessment, compound-specific radiocarbon, lignin phenols, *n*-alkanoic acid.

INTRODUCTION

Compound-specific radiocarbon (^{14}C) analysis (CSRA) is a powerful tool for studying the carbon cycle as it provides information about the sources and transport mechanisms of biomarker molecules. A major challenge in CSRA of biomarkers is the low abundance of these specific compounds in natural matrices (e.g. sediments and water) from which they are commonly extracted. This often requires CSRA to work with samples of small sizes (< 100 µgC). Recent improvements in the technology of accelerator mass spectrometry (AMS) permit the radiocarbon analysis of samples as small as ~1 µgC (Santos et al. 2007). However, small samples are very sensitive to biases caused by contaminating carbon (carbon of unknown isotopic composition and from unknown sources, defined as blank) that enters the samples during processing in the laboratory. For instance, the discrepancy

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between blank-uncorrected $F^{14}C$ value of a prepared standard (11 μgC) and its corresponding true $F^{14}C$ value might be as large as 0.229, and this discrepancy even increases as the sample size decreases (Hanke et al. 2017). Therefore, it is necessary to carefully assess and correct for the mass and ^{14}C content of the blank.

The preparation of samples for CSRA usually requires a series of complex procedures. An unknown amount of contaminant carbon of unknown $F^{14}C$ value might be introduced into the sample at any of these steps, such as during chemical extraction, isolation of pure compounds with preparative capillary gas chromatography (PCGC) or preparative-high performance liquid chromatography (prep-HPLC), preparation on vacuum line systems, and, in some cases, graphitization (Shah and Pearson 2007; Ziolkowski and Druffel 2009; Feng et al. 2013). Potential contamination sources include solvents, column bleed (from PCGC, prep-HPLC), carry-over and atmospheric carbon during combustion and vacuum line handling. Combined, these procedural blanks can be large enough to contribute a significant proportion of the mass of purified compound samples or even outweigh the target compound for ultra-small mass samples (Shah and Pearson 2007). The $F^{14}C$ value of the analyzed samples will significantly deviate from the true values of the target compounds without the proper assessment and correction of procedural blank, which will potentially lead to erroneous interpretation of the biogeochemical characteristics or cycling of the biomarker compounds. Therefore, the assessment of procedural blanks, i.e. the determination of $F^{14}C$ and the mass of the procedural blank ($F^{14}C_{blank}$, m_{blank}), is critical for reporting accurate radiocarbon composition.

Several studies have used various approaches to quantify the procedural blank and have attempted to identify the sources of the contaminating carbon. Shah and Pearson (2007) measured the masses of procedural blanks from different volumes of effluent from a prep-HPLC system (no sample added) and found masses of the procedural blank to be correlated to the prep-HPLC effluent volumes, which suggests that the procedural blank introduced during the isolation of compounds would vary in proportion to the mass of sample (the larger size samples require larger effluent volume). They also observed that the blank introduced from combustion is constant and there are some additional blanks introduced during other preparation steps in addition to prep-HPLC and combustion that are difficult to identify. Ziolkowski and Druffel (2009) have analyzed the mass and $F^{14}C$ of the eluted procedural blank from repeated dry injections (no solvent injected) on PCGC to directly evaluate the blank introduced from the PCGC separation step. An indirect method of determining the $F^{14}C$ of PCGC isolated size-series of paired standard compounds (one modern, one fossil) has also been used to calculate the masses of modern and fossil blanks introduced during the PCGC step. Ziolkowski and Druffel (2009) have shown that the direct and indirect methods agree in the assessment of the mass and $F^{14}C$ of procedureal blank and half of the procedural blank is introduced before PCGC isolation and likely from the chemical extraction step. In the study of Tao et al. (2015), the authors added modern and fossil standards of known $F^{14}C$ values into solvent blanks and used the deviation between the measured and known $F^{14}C$ values to indirectly assess the amount of modern and fossil blanks. Santos et al. (2010) proposed an approach to consider the amount of modern and fossil procedural blanks as integrated components which are a combination of all potential sources. Hanke et al. (2017) separated the procedural blank into ^{14}C-depleted and modern components and varied their masses to obtain the best m_{blank} and $F^{14}C_{blank}$ by chi-square fitting.

As stated above, preparing samples for CSRA involves many steps. Although it is possible to quantify the mass and $F^{14}C$ value of extraneous carbon from each step (Hanke et al. 2017) and

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potentially helpful when attempting to minimize the procedural blank, such work can be very time consuming depending on the preparation steps included, which may further increase the workload required for CSRA analysis. In addition, a detailed assessment of contaminating carbon contributions from each step will further complicate the error propagation during the correction for the procedural blank and introduces additional large uncertainties into the final $F^{14}C$ data. Therefore, a simplified but precise approach for blank assessment, which integrates over all preparation steps and avoids the detailed determination of individual contaminant sources, is highly needed for CSRA analysis—especially for small samples.

Here, we present a protocol for blank assessment that is relatively easy to achieve without complicated calculation or labor-intensive laboratory procedures. It is based on existing methods (Donahue et al. 1990; Hwang and Druffel 2005; Santos et al. 2007) and advances them by the application of a Bayesian model to more accurately account for uncertainties. As a case study, we apply our method to two different biomarker compound classes (*n*-alkanoic acid and lignin phenols), both commonly targeted for CSRA, to test whether it is practical for different compounds and preparation procedurals.

BLANK ASSESSMENT

In our approach we neither focus on the extraneous carbon added through individual preparation steps, nor attempt to determine modern C and fossil C contamination separately. Instead, the procedural blank is considered integrally. This approach is based on a hypothesis stated in the studies of Hwang and Druffel (2005) and Santos et al. (2007) according to which the mass and $F^{14}C$ value of the integral procedural blank is generally constant per batch of samples handled with the same preparation protocol for a certain class of compounds. Relying on this assumption, the measured mass and $F^{14}C$ value of a processed sample consists of the pure compound of interest and the constant contaminant (blank). Thus, the measured mass (m) and $F^{14}C$ value of a processed sample can be described as Equation (1) and (2), respectively (Hwang and Druffel 2005).

$$m_{\text{sample}} = m_{\text{true}} + m_{\text{blank}} \tag{1}$$

$$F^{14}C_{\text{sample}} = F^{14}C_{\text{true}} \times \left(\frac{m_{\text{true}}}{m_{\text{sample}}}\right) + F^{14}C_{\text{blank}} \times \left(\frac{m_{\text{blank}}}{m_{\text{sample}}}\right) \tag{2}$$

Where m_{sample}, m_{true} and m_{blank} refer to the mass of carbon of the processed sample, the pure compound and the procedural blank, respectively. $F^{14}C_{\text{sample}}$, $F^{14}C_{\text{true}}$ and $F^{14}C_{\text{blank}}$ are the $F^{14}C$ values of a processed sample, the pure compound and the procedural blank, respectively. Equation (2) can be rearranged to show the relation between $F^{14}C_{\text{sample}}$ and m_{sample}:

$$F^{14}C_{\text{sample}} = (F^{14}C_{\text{blank}} \times m_{\text{blank}} - F^{14}C_{\text{true}} \times m_{\text{blank}}) \times \frac{1}{m_{\text{sample}}} + F^{14}C_{\text{true}} \tag{3}$$

Except for m_{sample}, the other terms in Equation (3) are constant when using differently sized aliquots of the same material. Therefore, Equation (3) shows a linear relation between $F^{14}C_{\text{sample}}$ and $1/m_{\text{sample}}$ (Donahue et al. 1990; Hwang and Druffel 2005; Shah and Pearson 2007). The intercept ($F^{14}C_{\text{true}}$) is the $F^{14}C$ value of the pure compound and the slope (a) is defined as:

$$a = F^{14}C_{\text{blank}} \times m_{\text{blank}} - F^{14}C_{\text{true}} \times m_{\text{blank}} \tag{4}$$

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This shows the effect of the procedural blank on the measured $F^{14}C_{sample}$ as a function of the sample size (m_{sample}). It allows the procedural blank to be assessed graphically when determining the $F^{14}C_{sample}$ of several aliquots (of different size) of two standard materials, with known $F^{14}C_{true}$ but different values ($F^{14}C_{true1}$ and $F^{14}C_{true2}$), ideally one modern and one fossil standard. We can correlate the $F^{14}C_{sample}$ to $1/m_{sample}$ resulting in two regression lines with two slopes (a_1 and a_2), which can be used to derive the m_{blank} from their point of intersection:

$$m_{blank} = \frac{a_1 - a_2}{(F^{14}C_{true2} - F^{14}C_{true1})} \tag{5}$$

The $F^{14}C_{blank}$ can then be calculated as:

$$F^{14}C_{blank} = \frac{a_1}{m_{blank}} + F^{14}C_{true1} \quad or \quad F^{14}C_{blank} = \frac{a_2}{m_{blank}} + F^{14}C_{true2} \tag{6}$$

The chosen standards should contain the same or at least similar biomarker compounds as the set of "real" samples which is intended to be blank corrected. The standards and "real" samples should be processed using identical protocols. The range of chosen sample sizes (m_{sample}) for the standards should include the mass-range covered by the real sample-set and extend across the entire mass range covered by the method, e.g., 10–100 μgC.

For the blank-correction of real samples, robust estimates of the uncertainties in $F^{14}C_{blank}$ and m_{blank} are critical. In the approach described above $F^{14}C_{blank}$ and m_{blank} are afflicted with uncertainties stemming from the linear fit and from the measurements of the mass and $F^{14}C$ values of the different sized standards. Both of these should be considered when calculating the intersection point. In earlier studies applying the linear regression for the blank assessment, the standard error of the slopes of the regression lines (Hwang and Druffel 2005) or the correlation coefficient r^2 of the regression (Shah and Pearson 2007) were used to assess $\sigma(F^{14}C_{blank})$ and $\sigma(m_{blank})$. However, these approaches only account for the uncertainties introduced by the linear fit and do not consider the measurement uncertainties. Accordingly, we introduce a Bayesian model that includes error models for response and predictor variables taking both sources of uncertainty into account. This method allows for easy numerical estimation of the bivariate distribution of the intersection of the two regression lines (from which m_{blank} and $F^{14}C_{blank}$ are inferred) using the posterior sample of the distribution of the model parameters. The statistical model was written in the Stan language (Carpenter et al. 2017) and was fitted using the RStan package (Stan Development Team 2018) for Rstudio 1.1383 (R Core Team 2017). The values of $1/m_{blank}$ and $F^{14}C_{blank}$ (the intersection point) were constrained to be positive. Weak half-normal priors (mean = 0, sd = 10) were placed on the regression slopes, with the fossil slope constrained to be positive and the modern slope negative. In some special cases where the $F^{14}C_{blank}$ is higher than the $F^{14}C_{true}$ of the modern standard, the constraint on the modern slope should be removed. When available, $F^{14}C_{true}$ values for the standards were used to place an informative prior on the value of the intercept ($F^{14}C$ value at $1/m = 0$). Three chains of the fitting process were run for 5000 iterations and checked for convergence visually and with the Rhat statistics (Gelman and Rubin 1992). The output from the Bayesian model is the "posterior distribution," which consists of a matrix of parameter estimates based on 7500 iterations, 2500 from the second half of each chain. Each iteration provided one paired estimate of $F^{14}C_{blank}$ and $1/m_{blank}$. The median absolute deviation (MAD) is used as a robust measure of uncertainty for error propagation because the intersection is the ratio of the differences in slopes and intercepts, whose distribution has long tails. For normally distributed variables, the expected value of MAD is equal to

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the standard deviation. The script and the necessary Stan-code file are provided in the supplementary material along with diagnostic plots of the model fit.

CASE STUDIES

We applied this approach to two groups of biomarkers, i.e. *n*-alkanoic acids (lipid biomarkers) and lignin phenols. For the blank assessment of radiocarbon analysis on lipid biomarkers, *n*-hexadecanoic acid (n-$C_{16:0}$ alkanoic acid) from apple peel collected in 2013 ($F^{14}C$ value of bulk OC = 1.031 ± 0.001) was used as modern standard. A commercial *n*-triacontanoic acid (n-$C_{30:0}$ alkanoic acid; Sigma-Aldrich Prod. No. T3527-100MG, LOT 018K3760) of known $F^{14}C$ value (0.002 ± 0.001) (Rethemeyer et al. 2013) as well as *n*-hexacosanoic acid (n-$C_{26:0}$ alkanoic acid) and *n*-octacosanoic acid (n-$C_{28:0}$ alkanoic acid) extracted from Messel Shale (immature Eocene oil shale, $F^{14}C$ value of bulk OC = 0.0003 ± 0.0002) were used as fossil standards.

For the blank assessment of radiocarbon analysis on lignin phenols, vanillin extracted from woodchips collected in the wood workshop of University of Bremen in 2010 was used as the modern standard and the commercial standard ferulic acid (Sig-Aldrich, Prod. No.12,870-8, Lot STBB6360) of known $F^{14}C$ value (0.0002 ± 0.0004) was used as fossil standard.

The handling of purified standards for ^{14}C analysis was described in the study of Winterfeld et al. (2018) and Sun et al. (submitted for publication). Briefly, the procedure involves flame-sealing the standards with CuO in a vacuum line system and combustion to CO_2 that was purified and transferred to glass ampoules in the next step on the same vacuum line system. The ^{14}C of these standards was analyzed as gaseous samples using the miniaturized radiocarbon dating system (MICADAS) at the Laboratory of Ion Beam Physics, ETH Zürich (Ruff et al. 2007).

Case Study I: *n*-Alkanoic Acid Samples—Methods and Results

To collect sufficient n-$C_{16:0}$ and n-$C_{26:0–28:0}$ alkanoic acid from standard material to permit isolation of multiple aliquots, about 2 g dried apple peel and about 10 g dried and homogenized Messel Shale were Soxhlet-extracted with dichloromethane (DCM): methanol (MeOH) 9:1 (v/v) at 60°C for 48 hr and further processed by the method described in Mollenhauer and Eglinton (2007). Additionally, asphaltene precipitation was performed with the total lipid extract of the Messel Shale according to the protocol described in Weiss et al. (2000). The dried total lipid extracts were saponified with 0.1 N potassium hydroxide (KOH) in MeOH:H_2O 9:1 (v/v) at 80°C for 2 hr. After the extraction of neutral compounds by *n*-hexane, the solution was acidified to pH = 1. The acid fraction was extracted by DCM. Approximately 2 mg of the commercial standard n-$C_{30:0}$ alkanoic acid was processed following the same procedure as the extracted acid fraction from this step onwards. The acid fractions and n-$C_{30:0}$ alkanoic acid were then methylated with MeOH of known $F^{14}C$ value (0.0008 ± 0.0001) to corresponding *n*-alkanoic acid methyl esters in 5% HCl under N_2 atmosphere at 50°C overnight. The *n*-alkanoic acid methyl esters were extracted into *n*-hexane and further eluted with DCM:*n*-hexane 2:1 (v/v) through silica gel column chromatography. The targeted n-$C_{16:0}$, n-$C_{26:0}$, n-$C_{28:0}$ and n-$C_{30:0}$ alkanoic acid methyl esters were purified and collected by preparative capillary gas chromatography (PCGC) following the methods described by Eglinton et al. (1996) and Kusch et al. (2010). The injection volume was 5 µl, and ~25–120 repeated injections were conducted to collect sufficient mass of individual standard approximately ~22–151 µgC. This covers a reasonable range of

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Table 1 The measured m_{sample} and $F^{14}C_{sample}$ of standard compounds for the blank assessment for *n*-alkanoic acid methyl ester. $F^{14}C$ of unprocessed compounds are adopted from bulk organic carbon of Messel Shale and apple peel. Errors are given in 1σ.

Standard compound	Lab no.	$m_{sample} \pm \sigma$ (μgC)	$F^{14}C_{sample} \pm \sigma$ ($F^{14}C_{sample}$)
Fossil standard			
Unprocessed *n*-$C_{28:0}$ alkanoic acid	ETH no. 64615.1.1	n.a.	0.0003 ± 0.0002
Unprocessed *n*-$C_{28:0}$ alkanoic acid methyl ester*			0.0003 ± 0.0002
Processed *n*-$C_{30:0}$ alkanoic acid methyl ester	ETH no. 64819.1.1	89.00 ± 4.45^{2015S}	$0.0400 \pm 0.0016^{2015S}$
	ETH no. 68295.1.1	63.00 ± 3.15^{2015S}	$0.0568 \pm 0.0028^{2015S}$
	ETH no. 59361.1.1	24.00 ± 1.2^{2014W}	$0.1453 \pm 0.00338^{2014W}$
Processed *n*-$C_{28:0}$ alkanoic acid methyl ester	ETH no. 74341.1.1	81.00 ± 4.05^{2017}	0.0220 ± 0.0011^{2017}
	ETH no.74344.1.1	32.00 ± 1.60^{2017}	0.0582 ± 0.0018^{2017}
	ETH no. 68298.1.1	23.00 ± 1.15^{2017}	0.1833 ± 0.0030^{2017}
Processed *n*-$C_{26:0}$ alkanoic acid methyl ester	ETH no. 74342.1.1	108.00 ± 5.40^{2017}	0.0685 ± 0.0028^{2017}
	ETH no. 74343.1.1	75.00 ± 3.75^{2017}	0.0625 ± 0.0019^{2017}
Modern standard			
Unprocessed *n*-$C_{16:0}$ alkanoic acid	ETH no. 64615.1.1	n.a.	1.0311 ± 0.0038
	ETH no. 70188.1.1	n.a.	1.0263 ± 0.0026
	ETH no. 70122.1.1	n.a.	1.0279 ± 0.0026
Mean	n.a.	n.a.	1.0284 ± 0.0030
Unprocessed *n*-$C_{16:0}$ alkanoic acid methyl ester*	n.a.	n.a.	0.9705 ± 0.0036
Processed *n*-$C_{16:0}$ alkanoic acid methyl ester	ETH no. 59306.1.1	151.00 ± 7.55^{2014W}	$0.9650 \pm 0.0078^{2014W}$
	ETH no. 74369.1.1	136.00 ± 6.80^{2017}	0.9670 ± 0.0061^{2017}
	ETH no. 64822.1.1	119.00 ± 5.95^{2015S}	$0.9960 \pm 0.0015^{2015S}$
	ETH no. 64821.1.1	67.00 ± 3.35^{2015S}	$0.9442 \pm 0.0088^{2015S}$
	ETH no. 74368.1.1	44.00 ± 2.20^{2017}	0.9594 ± 0.0068^{2017}
	ETH no. 59307.1.1	22.00 ± 1.10^{2014W}	$0.9013 \pm 0.0083^{2014W}$

n.a.: not available. The superscript W and S refer to the data adopted from Winterfeld et al. (2018) and Sun et al. (submitted for publication). The superscript numbers represent the years when the standards were prepared and analyzed on AMS. * indicates the $F^{14}C$ of the alkanoic acid methyl ester calculated based on the $F^{14}C$ of corresponding unprocessed alkanoic acid and methanol (see Case Study I).

sample sizes, in which samples for CSRA may commonly occur (Table 1). The purity of these standards was checked by injecting a small aliquot of collected standards to a gas chromatograph coupled to a flame ionization detector (GC-FID). The purified *n*-alkanoic acid methyl esters were flame-sealed on a vacuum line system with CuO (pre-combusted) and were subsequently combusted at 850°C for 5 hr to oxidize the compounds to CO_2. Afterwards, the CO_2 samples were purified (dried), and transferred into small glass ampoules on the vacuum line in order to prepare the samples for $F^{14}C$ analysis on AMS. The gas volume analyzed was determined on the AMS.

The measured m_{sample} and $F^{14}C_{sample}$ of the modern and fossil standards of this case study are listed in Table 1. For the blank assessment it is assumed that the true $F^{14}C$-values of the

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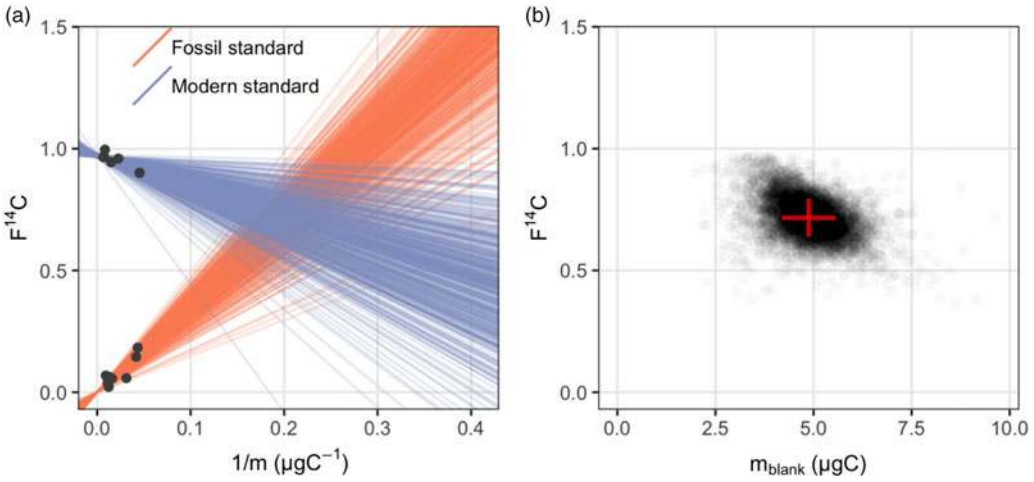

Figure 1 Procedural blank assessment for *n*-alkanoic acid methyl esters: (a) a sample of 500 regression lines from the posterior distribution give a visual check of the fitted Bayesian model; (b) the posterior distribution of masses and $F^{14}C$ values of the procedural blank.

unprocessed *n*-alkanoic acids are identical to the $F^{14}C$ values of bulk organic carbon of apple peel and Messel Shale, respectively. It has to be acknowledged that as described above, in the course of the isolation procedure in the laboratory, *n*-alkanoic acids were methylated to *n*-alkanoic methyl esters in order to facilitate gas chromatography (e.g. Wakeham et al. 2006). Therefore, CSRA data of the processed standards are obtained from the methyl esters and not from the pure *n*-alkanoic acids. The methylation means that $F^{14}C_{sample}$ is affected by the $F^{14}C$ of the added methyl-group ($F^{14}C_{methyl}$) next to the unknown blank. Hence, when determining the m_{blank} and $F^{14}C_{blank}$ as discussed above and shown in Figure 1, the methyl group of the processed n-$C_{16:0}$ and n-$C_{26:0\text{-}30:0}$ methyl esters affects the slope of the regression lines. As a result, this would count towards the unknown blank. We corrected for this effect by combining the $F^{14}C_{methyl}$ value, with the $F^{14}C_{true}$ of the modern and fossil standard (bulk values of apple peel and Messel Shale) by isotopic mass balance to obtain the $F^{14}C_{true}$ of the respective unprocessed methyl esters. The calculated value is set as the intercept for the regression lines as indicated in Equation (3).

It appears in Figure 1a that both standards display significant linear relationships between their measured $F^{14}C_{sample}$ and $1/m_{sample}$ as expected according to Equation (3). Although the fossil standards include saturated *n*-alkanoic acid methyl esters with different chain lengths (n-C_{26}, n-C_{28} and n-C_{30}), their $F^{14}C_{sample}$ and $1/m_{sample}$ relationships are consistent. It is also worth noting that these fossil standards were actually processed at different times between 2014 and 2017. However, this did not influence the consistency in the linear relationship, which suggests that the procedural blank is relatively invariant with time. A sample from the posterior distribution of regression lines fitted with the Bayesian model is plotted in Figure 1a. Figure 1b shows the posterior distribution of masses and $F^{14}C$ values of the procedural blank, which are obtained from the pairwise intersection points of the regression lines. Using our Bayesian model, the m_{blank} and $F^{14}C_{blank}$ of the *n*-alkanoic acids and their uncertainties are estimated at $m_{blank} \pm \sigma(m_{blank})$ 4.88 ± 0.69 μgC and $F^{14}C_{blank} \pm \sigma(F^{14}C_{blank})$ 0.714 ± 0.077, respectively (Table 3).

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Table 2 Measured m_{sample} and $F^{14}C_{sample}$ of standard compounds for the blank assessment of lignin phenols (Sun et al., submitted for publication).

Standard compound	Lab no.	m_{sample} (μgC)	$F^{14}C_{sample} \pm \sigma$ ($F^{14}C_{sample}$)
Fossil standard			
Unprocessed ferulic acid	ETH no. 51004.1.1	n.a.	0.0002 ± 0.0004
Processed ferulic acid	ETH no. 68305.1.1	83.00 ± 4.15	0.0095 ± 0.0013
	ETH no. 68306.1.1	51.00 ± 2.55	0.0076 ± 0.0012
	ETH no. 68307.1.1	33.00 ± 1.65	0.0110 ± 0.0013
	ETH no. 68308.1.1	13.00 ± 0.65	0.0130 ± 0.0016
	ETH no. 68336.1.1	6.00 ± 0.30	0.1446 ± 0.0067
Modern standard			
Vanillin	ETH no. 68309.1.1	70.00 ± 3.50	1.2129 ± 0.0115
	ETH no. 68310.1.1	50.00 ± 2.50	1.2007 ± 0.0113
	ETH no. 68311.1.1	29.00 ± 1.45	1.2237 ± 0.0120
	ETH no. 68312.1.1	11.00 ± 0.55	1.1875 ± 0.0132
	ETH no. 68337.1.1	6.00 ± 0.30	1.1577 ± 0.0234

n.a.: not available.

Case Study II: Lignin Phenol Samples—Methods and Results

Vanillin from woodchips was extracted using the method of Goñi and Montgomery (2000). Briefly, about 10 g of woodchip were oxidized with copper oxide (CuO) and ferrous ammonium sulfate in de-aerated 2 N sodium hydroxide (NaOH) at 150°C for 90 min under a nitrogen (N_2) atmosphere in a CEM MARS5 microwave accelerated reaction system. After the oxidation, the supernatant was acidified to pH<1 and the reaction products were extracted into ethyl acetate. Approximately 3 mg of commercial standard ferulic acid were dissolved in ethyl acetate and processed as the extracted oxidation products according to the method of Feng et al. (2013).

Briefly, the extracts and the ferulic acid were both pre-cleaned with Supelclean ENVI-18 solid phase extraction (SPE) cartridges and eluted with acetonitrile. Subsequently, the vanillin from the extracts and ferulic acid were further isolated by LC-NH_2 SPE cartridges and were eluted into MeOH and MeOH:12 N HCl 95:5 (v:v), respectively. The vanillin and ferulic acid were extracted from their elution with ethyl acetate and re-dissolved in MeOH for purification on prep-HPLC. The vanillin was then purified with a Phenomenex Synergi Polar-RP column followed by a ZORBAX Eclipse XDB-C18 column. The ferulic acid was purified with the same columns but in reverse order. The specific elution conditions on the prep-HPLC system can be found in Feng et al. (2013). ~ 20 repeated injections were conducted to collect sufficient mass of individual standard, which was divided into a range of sample sizes (Table 1). The purity of the collected standards was checked by injecting a small aliquot of the standard to GC-FID. All purified lignin phenolic compounds were flame-sealed with CuO on a vacuum line and were combusted to form CO_2, which was subsequently purified and transferred to smaller glass ampules on the vacuum line system.

As is the case of blank assessment for *n*-alkanoic acid methyl esters, the measured m_{blank} and $F^{14}C_{blank}$ of a range of different sized modern and fossil lignin phenolic standards are listed in Table 2. The $F^{14}C$ value of pure ferulic acid was measured as graphite target and assumed to be

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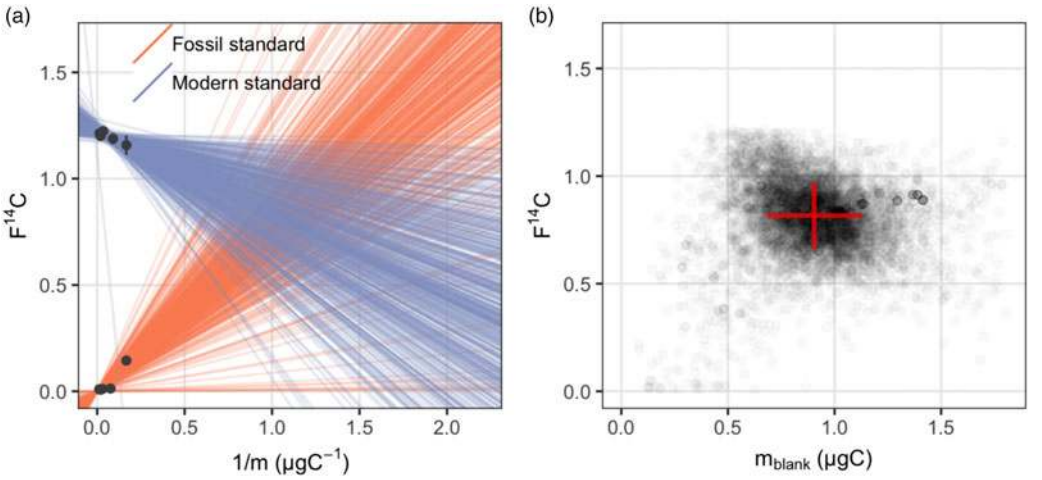

Figure 2 Procedural blank assessment for lignin phenols: (a) a sample of 500 regression lines from the posterior distribution give a visual check of the fitted Bayesian model; (b) the posterior distribution of masses and $F^{14}C$ values of the procedural blank.

Table 3 Estimated values of m_{blank} and $F^{14}C_{blank}$.

Blanks	Parameter	Mean	SD	Median	MAD
Blank of *n*-alkanoic acid	$F^{14}C_{blank}$	0.716	0.083	0.714	0.077
	m_{blank} (μgC)	4.898	0.746	4.881	0.691
Blank of lignin phenols	$F^{14}C_{blank}$	0.809	0.166	0.813	0.155
	m_{blank} (μgC)	0.927	0.291	0.905	0.229

the $F^{14}C_{true}$, which is set as the intercept for the regression line of the fossil standard. Note that the exact $F^{14}C$ value of wood chips from which the vanillin was extracted is not available, therefore the intercept of the regression line of modern standard ($F^{14}C_{true}$) cannot be defined. Similar to the lipid standards, the measured $F^{14}C_{sample}$ of both vanillin and ferulic acid are linearly related to the corresponding $1/m_{sample}$ (Figure 2a). This suggest that the assumption of a constant procedural blank is also valid for the purification of lignin phenolic compounds. The posterior distribution of the masses and $F^{14}C$ values of the procedural blank from the Bayesian model is shown in Figure 2b. The m_{blank} and $F^{14}C_{blank}$ value of the procedural blank during CSRA of lignin phenolic compounds are estimated at $m_{blank} \pm \sigma(m_{blank})$ 0.90 ± 0.23 μgC and $F^{14}C_{blank} \pm \sigma(F^{14}C_{blank})$ 0.813 ± 0.155, respectively (Table 3).

CASE STUDIES—DISCUSSION

For our two case studies, we are able to obtain statistically robust estimates of the mass and $F^{14}C$ value of the procedural blank (i.e. small uncertainties in both variables), despite requiring a long extrapolation of the regression lines to the intersection point. This also suggests that much smaller uncertainties can be obtained if small sized samples with masses close to the intersection point (mass of the blank) are available for the assessment of blanks because

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this will shorten the extrapolation distance. Therefore, the smallest sample sizes of the set of standards should be as small as possible to achieve a relatively short extrapolation distance, which will produce better estimates of m_{blank} and $F^{14}C_{blank}$.

Our results of the mass and $F^{14}C$ values of procedural blanks for lignin phenolic compounds by the Bayesian model agree well with the one obtained by least-square model (0.90 ± 0.18 μgC with $F^{14}C$ of 0.814 ± 0.407, Haghipour et al. 2018). The mass and $F^{14}C$ values of procedural blanks for *n*-alkanoic acid methyl esters and lignin phenolic compounds can be further applied to correct for the $F^{14}C$ values of the real samples. The $F^{14}C$ of the procedural blank (0.714 ± 0.077 or $\Delta^{14}C = -292 \pm 71‰$) for *n*-alkanoic acid methyl esters is similar to the procedural blank determined in the study of Tao et al. (2015) ($\Delta^{14}C = -325 \pm 129‰$), in which a similar sample preparation protocol was used. In Tao et al. (2015), the mass of the combined procedural blank was determined to be 1.3 ± 0.2 μgC per 30 PCGC injections, which means that these authors assumed the procedural blank varies with the sample size rather than a constant procedural blank.

The larger procedural blank for *n*-alkanoic acid methyl esters (4.88 ± 0.69 μgC) means that for this case, the results of small size samples (e.g. <15 μgC) are meaningless due to a high proportion of contaminant carbon (~30%). Compared to the preparation process of *n*-alkanoic acid methyl esters, our preparation of CSRA for lignin phenols introduced a lower amount of procedural blank (0.90 ± 0.23 μgC). Although the masses of procedural blank in preparation of lignin phenols and *n*-alkanoic acid methyl esters are different, their $F^{14}C$ values are identical within errors. This implies that the blank introduced by the two different protocols has the same composition and source but varies in size. The general difference in the preparation for these two types of compounds lies in almost every step, i.e., chemical extraction, cleaning, isolation methods. For example, it includes Soxhlet extraction, purification with PCGC, flame-sealing on the vacuum line and combustion for *n*-alkanoic acid methyl esters and alkaline CuO oxide digestion combined with solvent extraction followed by isolation with prep-HPLC and all the vacuum line handling and combustion for lignin phenols. As such, it is reasonable to assume that the different masses of the blank are associated with the different preparation procedures.

According to the results of these two case studies, our approach of blank assessment is successfully applied for *n*-alkanoic acid methyl esters and lignin phenols that require different isolation methods. It demonstrates that this blank assessment method can further be applicable for other compounds and various preparation protocols. Unlike the methods considering modern or fossil procedural blank separately and assessing contamination introduced from different preparation steps, our method is not difficult to achieve and reduces the complexity in the calculation of uncertainty. Therefore, this method has the potential to serve as a simple and widely applied approach for blank assessment. We propose to routinely conduct blank assessment for different batches of samples and different compounds-classes to ensure the accuracy and precision of $F^{14}C$ values of real samples of purified organic compounds, especially of small sizes (<100 μgC).

CONCLUSION

Based on our methods of blank assessment, we observe that our preparation protocol of radiocarbon analysis of *n*-alkanoic acid and lignin phenols will produce 4.88 ± 0.69 μg of extraneous carbon with $F^{14}C$ of 0.714 ± 0.077 and 0.90 ± 0.23 μg of extraneous carbon with

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$F^{14}C$ of 0.813 ± 0.155, respectively. The $F^{14}C$ of the procedural blanks for both biomarkers are similar, but the mass of the procedural blank of *n*-alkanoic acid is five times larger than that for lignin. This discrepancy is probably due to different chemical cleaning, isolation methods and preparation on the vacuum line system thereby highlighting the necessity to conduct blank assessment for different compound classes and preparation procedures. The method proposed in this study is neither time consuming nor labor intensive; it is worth extending to other biomarkers and may also serve as a standardized method for blank assessment.

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SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit https://doi.org/10.1017/RDC.2019.108

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