An automated method for the determination of the TEX₈₆ and U₃₇^{K'} paleotemperature indices

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Abstract

The relative abundances of archaeal glycerol dialkyl glycerol tetraether (GDGTs) compounds (TEX₈₆) and alkenone lipids (U₃₇^{K'}) are commonly used paleoceanographic proxies for temperature. These proxies are analyzed by high-performance liquid chromatography (HPLC) and gas chromatography (GC) methods, respectively, and traditionally, the peaks corresponding to the individual compounds are integrated by hand. Here, we introduce a MATLAB software package called ORIGAmI (ORganIc Geochemistry peAk Integration) that allows for automated, reproducible determination of these indices and quantification of lipid concentrations. The method is based on modeling the peaks with individual Gaussian curves. Comparisons with manually integrated data demonstrate that the method is accurate and precise. Furthermore, automating the peak detection and quantification process reduces both analysis time and user error.

Keywords: TEX₈₆, U₃₇^{K'}, paleotemperature proxies, Gaussian peak detection

1. Introduction

The estimation of past changes in ocean temperature is an important component of paleoclimatology, providing insights into fundamental aspects of the Earth's climate system. Over the past thirty years, several paleothermometers employing the distribution of biomarkers have been developed, relying

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on the general principle that micro-organisms adjust the structure of their cell membrane lipids in response to environmental temperature (Ray et al., 1971; Kita et al., 1973; Nozawa et al., 1974). Amongst these are the relative abundance of tri- and di-unsaturated alkenone lipids produced by photosynthetic haptophyte algae such as *Emiliana huxleyi*: the so-called $U_{37}^{K'}$ proxy (Brassell et al., 1986). $U_{37}^{K'}$ is perhaps the most widely-used lipid paleothermometer, and has provided detailed sea-surface temperature (SST) histories throughout the Cenozoic (Herbert, 2003). More recently, another paleothermometer based on the relative cyclization of marine archaeal glycerol dialkyl glycerol tetraethers (GDGTs) was developed: the TetraEther index of 86 carbons (TEX₈₆, Schouten et al., 2002). In pelagic environments, isoprenoidal GDGTs are produced predominantly, although likely not exclusively (Lincoln et al., 2014), by ammonia-oxidizing Thaumarchaeota (Sinninghe Damsté et al., 2002). Laboratory mesocosm experiments show that Thaumarchaeota produce GDGTs with more rings at higher temperatures (Wuchter et al., 2004; Schouten et al., 2007a) and there is a strong empirical relationship between surface ocean temperatures and modern sedimentary TEX₈₆ (Schouten et al., 2002; Kim et al., 2008; Tierney and Tingley, 2014, 2015) enabling robust reconstruction of upper ocean temperatures. TEX₈₆ complements the $U_{37}^{K'}$ approach in that it is applicable over a wider range of temperatures (0–50°C for TEX₈₆ vs. 0–28°C for $U_{37}^{K'}$) and can be used farther back in geologic history; the oldest application to date falls within the middle Jurassic (Jenkyns et al., 2012).

$U_{37}^{K'}$ and TEX₈₆ can be analyzed rapidly and reliably by modern GC and HPLC techniques. $U_{37}^{K'}$ is typically analyzed via a GC coupled to a flame ionization detector (GC-FID, Herbert, 2003) whereas TEX₈₆ employs normal phase separation by HPLC and analysis by atmospheric-pressure chemical ionization (APCI) mass spectrometry (MS) (Hopmans et al., 2000; Schouten et al., 2007b). In both cases, the output is a chromatogram containing peaks corresponding to the compounds of interest. Although the proprietary instrument software usually provides automated peak detection, the algorithms cannot always adequately estimate background levels and peak areas, especially if the

background changes through the chromatogram and/or if peaks coelute with another compound. For both $U_{37}^{K'}$ and TEX_{86} , such circumstances are typical, leading users to resort to integrating the peaks of interest manually within the instrument software. While this approach may ensure more consistent integration when dealing with complex chromatograms, it is time-consuming, and becomes prohibitive when hundreds of samples must be analyzed in order to create a paleoclimatic time series. In addition, manual peak picking introduces subjectivity and user error to the results (e.g., [Dillon and Huang, 2015](#)).

Here, we present a software package called ORIGAmI (ORganIc Geochemistry peAk Integration) that allows for automatic integration and quantification of $U_{37}^{K'}$ and TEX_{86} in MATLAB. The primary functions `getAlkenones` and `getTEX86` employ the same peak detection algorithm, but handle inputs differently in accord with the different methods of analysis. We have also developed general functions `getGCareas` and `getHPLCareas` that can identify and integrate a user-defined set of peaks of interest. We describe our approach below, and then test the method against manually-integrated data to ensure accuracy and precision.

2. Methods

2.1. Laboratory methods

For $U_{37}^{K'}$ determination, three sediment samples from the North Atlantic (43.35°N, 60.208°W, modern in age), the subtropical eastern Atlantic (30.88°N, 10.63°W, dated to the last glacial maximum), and the tropical Gulf of Aden (11.955°N, 44.3°E, dated to last glacial maximum) respectively were extracted via Accelerated Solvent Extraction (ASE) at 100°C and 1500 psi using a mixture of CH_2Cl_2 :methanol (9:1). The samples were separated over LC-NH₂ into neutral (eluted with CH_2Cl_2 :isopropanol 2:1), acid (eluted with 4% acetic acid in CH_2Cl_2), and polar (100% methanol) fractions. The neutral and polar fractions were subsequently recombined and then separated over 5% deactivated silica gel into three fractions, eluting with hexane, CH_2Cl_2 , and methanol, re-

spectively. The CH_2Cl_2 fraction, containing the alkenones, was redissolved in ethyl acetate and analyzed by GC-FID using a Trace 1310 gas chromatograph (Thermo Fisher Scientific) equipped with a DB-1 column ($60\text{ m} \times 0.32\text{ mm} \times 0.1\text{ }\mu\text{m}$) and a programmable temperature vaporization (PTV) injector. The GC oven program (total runtime: 70 min) was: 60°C (held 2 min) to 270°C at $30^\circ\text{C}/\text{min}$, then to 310°C at $1^\circ\text{C}/\text{min}$ (hold 1 min), then to 325°C at $10^\circ\text{C}/\text{min}$ (hold 18.50 min). The PTV was operated in splitless mode (splitless time: 3 min) with a program of: 60°C (held 0.1 min) to 325°C at $5^\circ\text{C}/\text{sec}$, and a ramped pressure from 14.5–33.6 psi. Alkenones were detected by comparison of retention times to standards, and quantification was achieved using a stearyl stearate internal standard, added to the total lipid extract prior to the column purification steps. The three sediment samples were analyzed 20 times each in order to generate meaningful statistics. In addition, we measured a $\text{U}_{37}^{K'}$ time series on sediment core OCE 437-7 GC27 ($n = 61$) to further assess the effect of manual vs. automated peak integration methods on $\text{U}_{37}^{K'}$ determination. The age-depth model for OCE 437-7 GC27 was previously published (McGee et al., 2013).

For TEX_{86} determination, three sediment samples from the Arctic (71.2°N , 143°W , modern in age), the western North Atlantic (35.83°N , 74.83°W , modern in age) and the Caribbean (26.2°N , 77.7°W , modern in age) were extracted and prepped as described above. The methanol fraction from the silica gel separation, containing the GDGTs, was redissolved in hexane:isopropanol (99:1), filtered through a 0.45 micron PTFE filter and then analyzed on an Agilent 1260 HPLC coupled to an Agilent 6120 single quadrupole MS with an APCI source using the method of Schouten et al. (2007b). Briefly, separation was achieved using a Prevail Cyano column and a gradient from 90:10 (A:B) to 82:18 (A:B) where A = hexane and B = 9:1 hexane:isopropanol. Compounds were detected by single ion monitoring of their $[\text{M}+\text{H}^+]$ ions. Both manual and automatic integration was carried out on the individual extracted ion chromatograms. Quantification was achieved by addition of a synthetic C_{46} GDGT internal standard (Huguet et al., 2006) to the TLE prior to column separation, and calculation of

the relative response factor (RRF) by coinjection of the standard with a known amount of purified crenarchaeol, provided by Ann Pearson at Harvard University as part of the “GDGT standards project.” As with the $U_{37}^{K'}$ determination, the three sediment samples were analyzed 20 times each. In addition, we analyzed a TEX_{86} time series from sediment core P178-15P ($n = 25$) to further compare manual and automatic TEX_{86} determination. The age-depth model for P178-15P was previously published (Tierney and deMenocal, 2013).

2.2. ORIGAmI software

2.2.1. System requirements

ORIGAmI has been tested on MATLAB 2013b and higher on Apple OS X 10.9, 10.10, and 10.11 and Windows 7 platforms. ORIGAmI requires MATLAB’s Signal Processing Toolbox and Curve Fitting Toolbox.

2.2.2. Data export and import

For TEX_{86} , extracted ion chromatograms for each ion of interest (m/z 1302, m/z 1300, m/z 1298, m/z 1296, m/z 1292, m/z 1050, m/z 1036, m/z 1022) were exported from Agilent Chemstation to individual .txt files using a macro `txtfile.mac`, which is included in the ORIGAmI package (see Supplementary Materials). For $U_{37}^{K'}$, chromatograms were manually exported from XCalibur software to a single .csv file. ORIGAmI then automatically imports the data into MATLAB for analysis.

2.2.3. Peak detection

After importing, we smooth the data using Whittaker’s penalized least-squares smoothing algorithm (Eilers, 2003) to reduce noise. We then determine which peaks correspond to the compounds of interest. In chromatography, the retention times of the peaks may change slightly from sample to sample, but the distance between peaks remains relatively constant. The user can therefore define an array of expected retention times, $\mathbf{T}$, of length k , where k = number of compounds:

$$\mathbf{T} = \begin{pmatrix} T_1 & T_2 & \dots & T_k \end{pmatrix} \quad (1)$$

and our program determines which peaks most closely match the distance between these retention times,

$$\begin{aligned}\Delta\mathbf{T} &= \begin{pmatrix} \Delta T_1 & \Delta T_2 & \dots & \Delta T_{k-1} \end{pmatrix} \\ &= \begin{pmatrix} T_2 - T_1 & T_3 - T_2 & \dots & T_k - T_{k-1} \end{pmatrix}\end{aligned}\quad (2)$$

If the user does not define the array $\mathbf{T}$, the program uses a pre-defined array based on a sample file.

120 The compounds of interest may be either local maxima or shoulders. Shoulders are the locations in the trace where the first derivative is approximately zero, but does not change signs. To find local maxima, we use MATLAB’s built-in `findpeaks` function. To find shoulders, we calculate the first derivative of the data, and smooth the derivative using Whittaker’s penalized least-squares
125 smoothing algorithm to once again reduce noise. We rescale the derivative so that the maximum of the absolute value of the derivative is 1. We then find all points where the absolute value of the derivative is less than 0.01. Any such points that are immediately adjacent to each other form a cluster. If the sign of the derivative of the cluster changes more than once, we disregard that cluster
130 as noise. In each remaining cluster, the point with the derivative closest to zero is the peak for that compound.

To improve the speed of the calculation, we only include peaks that are likely to match the compounds of interest. Thus, we require that the peaks must be at least 1% of the height of the tallest peak in the data, $y_{\max}$, and they must occur at least 0.01 minutes apart. The peaks must also fall within one of the windows of length w that are centered around each of the theoretical retention times. The user can define w or use the default value of 2 minutes. We then find the set, $\mathbf{t}$, of all k -combinations of the retention times of these peaks:

$$\mathbf{t} = \begin{pmatrix} t_{11} & t_{12} & \dots & t_{1k} \\ t_{21} & t_{22} & \dots & t_{2k} \\ \vdots & \vdots & & \vdots \\ t_{j1} & t_{j2} & \dots & t_{jk} \end{pmatrix}\quad (3)$$

The set $\Delta \mathbf{t}$ is the difference between adjacent retention times for all k -combinations.

If we define $S(n)$ for the n th combination as the sum of the square of the differences between the theoretical relative retention times and the relative retention times of the combination,

$$S(n) = \sum_{i=1}^{k-1} (\Delta T_i - \Delta t_{ni})^2 \quad (4)$$

then the combination with the smallest value of S is the most likely match to the theoretical retention times.

Once the peaks of interest are identified, we must determine the boundaries of each peak. Because each peak is approximately Gaussian, the first derivative of the data approaches zero on either side of a peak, such that

$$\lim_{x \rightarrow -\infty} \frac{dy}{dx} = 0^+ \quad \lim_{x \rightarrow \infty} \frac{dy}{dx} = 0^-, \quad (5)$$

135 with a positive value to the left of the peak and a negative value to the right of the peak. We can find the boundaries of a peak by identifying where the first derivative is below (above) a certain threshold on the left (right) side of the peak. We smooth the derivative using the Whittaker's penalized least-squares smoother and rescale it to have a maximum absolute value of 1. The left (right)
140 boundary is the point closest to the peak that has a derivative less than 0.005 (greater than -0.005). We also explicitly check for local minima in y between these boundaries and the peak. If any local minima exist in this region, they form the new boundary on that side of the peak. For peaks that are shoulders, the trace increases on one side of the shoulder and decreases on the other.
145 When we fit shoulders, we only include the data between the shoulder and the boundary on the decreasing side.

We then fit a Gaussian function to each peak:

$$Y(X) = a \exp \left(-\frac{(X - b)^2}{c^2} \right) \quad (6)$$

where Y is the Gaussian fit, X is the array of retention times, a is the height of the peak, b is the retention time of the center of the peak, and $c = \sqrt{2}\sigma$,

where σ is the standard deviation. We use MATLAB’s built-in curve fitting
 150 library with the conditions that $0 < a < 2y_{\max}$, $0 < b < 2x_{\max}$, where $x_{\max}$
 is the highest retention time of the trace, and $0 < c < 5$. If the maximum
 value of the Gaussian fit is more than 5% larger than $y_{\max}$, or if the R-squared
 value of the fit is less than 0.9, we refit the data with stricter conditions that
 $0 < a < 1.05y_{\max}$, $0 < b < x_{\max}$, and $0 < c < 1$.

155 Occasionally, two peaks co-elute (for example, C_{38:2 et} and C_{38:2 me} in fig.
 1). When these peaks are integrated manually using the instrument software,
 typically the co-eluting peaks are split into separate peaks by dividing them
 down the middle at the minimum between the two peaks. When calculating
 the area of a co-eluting peak this way, the instrument software ignores the
 160 overlapping tails. In the ORIGAmI software, the co-eluting peaks are modeled
 as individual peaks. When we calculate the area of the Gaussian curves for
 each peak, we include the tails that would otherwise be ignored. While the
 area of overlap for the co-eluting peaks is included in both peak areas, it is
 determined by the Gaussian fit, which in theory models the peak as if it were
 165 not co-eluting. This leads to substantially more accurate peak integration than
 manual methods.

We numerically approximate the area under the Gaussian curve using the
 trapezoidal method. For GDGTs, the TEX₈₆ value is calculated according to
 the definition:

$$\text{TEX}_{86} = \frac{\text{GDGT-2} + \text{GDGT-3} + \text{Cren}'}{\text{GDGT-1} + \text{GDGT-2} + \text{GDGT-3} + \text{Cren}'} \quad (7)$$

where GDGTs 1–3 are the areas of the peaks of compounds containing 1–
 3 cyclopentyl moieties, and Cren’ is the area of the regioisomer of crenar-
 chaeol. We also calculate the BIT (Branched and Isoprenoidal Tetraether) index
 170 (Hopmans et al., 2004) which is defined as:

$$\text{BIT} = \frac{\text{brGDGT-1} + \text{brGDGT-2} + \text{brGDGT-3}}{\text{brGDGT-1} + \text{brGDGT-2} + \text{brGDGT-3} + \text{Crenarchaeol}} \quad (8)$$

where brGDGTs 1–3 are the three major branched GDGTs found in marine

sediments with 4, 5, and 6 methyl groups, respectively.

For alkenones, the $U_{37}^{K'}$ value was calculated using the definition:

$$U_{37}^{K'} = \frac{C_{37:2}}{C_{37:3} + C_{37:2}} \quad (9)$$

where $C_{37:2}$ and $C_{37:3}$ are the areas of the di- and tri- unsaturated C_{37} alkenones.

We also calculated the $U_{38_{et}}^K$ and $U_{38_{me}}^K$ indices, which are identical to the $U_{37}^{K'}$
 175 index except that they use the $C_{38:3et}$, $C_{38:2et}$ and $C_{38:3me}$, $C_{38:2me}$ compounds respectively.

The output for `getTEX86` includes the list of the folders (each corresponding to a sample) that were analyzed, along with the corresponding files (individual extracted ion chromatograms as .txt files) in each folder, the areas of the peaks,
 180 the retention times of each peak, and the TEX_{86} and BIT values for each sample. The output for `getAlkenones` includes the list of files (sample GC traces) that were analyzed, as well as the areas and retention times of the peaks, and the $U_{37}^{K'}$, $U_{38_{et}}^K$, and $U_{38_{me}}^K$ values for each file. Concentrations of GDGTs and alkenones are calculated post hoc, using the peak areas of the internal standard and the
 185 target compounds, the amount of standard added, and the relative response factor (for GDGTs). Concentrations of alkenones are expressed as $U_{37}^{K'}$ "total" and U_{38}^K "total," which represents the combined concentration of the tri- and di-unsaturated C_{37} and C_{38} alkenones, respectively.

For both `getTEX86` and `getAlkenones`, the user may choose whether or not
 190 to display the chromatogram(s) and the integrated peaks.

3. Results and discussion

Figure 1 illustrates how the `getAlkenones` program evaluates a sample GC trace. In this example, the $C_{37:3}$ alkenone is small and partly co-elutes with another compound at ~ 41 minutes. Whereas manual integration would require
 195 truncating part of the peak, therefore increasing the $U_{37}^{K'}$ value, `getAlkenones` can identify and model the area of $C_{37:3}$ as a complete Gaussian peak. Generally speaking, we found that the peak detection algorithm we employ can correctly

model a peak in spite of co-elution unless the co-eluting peak elutes so closely to the target compound that the boundary between the two peaks becomes
200 difficult to detect from the first derivative. In this case, further sample clean-up would be required to properly identify and integrate the peak, as would also be the case for manual integration.

Figure 2 shows how `getTEX86` evaluates a sample HPLC trace containing GDGTs. Once again, the biggest differences between the automated and manu-
205 ally integrated peaks areas occur for peaks that have co-eluting shoulders. While the manual method excludes the tail of the peak where it starts to co-elute with another compound, the automated method includes this tail. In some cases, this may induce differences in the calculated TEX_{86} value, although in this particular example, the difference is minimal because the proportion of the peaks
210 that would be excluded by the manual integration method is relatively small.

To rigorously assess the ability of the ORIGAmI package to integrate alkenones and GDGTs, we conducted repeat analyses of a set of sediment samples spanning different marine environments and water temperatures. Figure 3 shows box plots of the TEX_{86} and BIT values for sediment samples from the Arctic, North
215 Atlantic, and Caribbean region that represent relatively cool, temperate, and warm ocean temperatures, respectively. Figure 3 also shows the concentrations of isoGDGTs and brGDGTs values for the Arctic and North Atlantic samples; the Caribbean sample lacked an internal standard, so concentrations were not calculated. We compared the values from manual integration and automated
220 integration using Welch’s t-test and the F test for equality of variances (Tables 1 and 2). For TEX_{86} , we could not reject the null hypothesis for the Arctic or North Atlantic samples that the automated and manual means are the same. We could reject the null hypothesis for the Caribbean sample, but the automated TEX_{86} value for the Caribbean sample is only 0.004 lower than the manually
225 integrated value, which is similar to the typical analytical precision for duplicate or triplicate analyses via HPLC-MS (Schouten et al., 2007b). For BIT, all samples had different means for manual and automated integration (Table 1), and the BIT values using `getTEX86` were systematically higher than the manu-

ally integrated values (Fig. 3). We attribute this difference to the fact that the
 230 branched GDGTs have many co-eluting peaks, and this results in smaller peak
 areas during manual integration as more area is excluded (cf. the alkenone
 example in Fig. 1). Even so, the differences in BIT are small (0.001–0.007)
 and generally similar to the analytical precision (0.004). For concentrations of
 isoGDGTs and brGDGTs, the automated integration consistently gave higher
 235 results, due to the fact that the automated integration includes more area for
 co-eluting peaks (Table 2).

All samples had the same variance for automated and manual values of
 TEX₈₆, concentrations of isoGDGTs, and concentrations of brGDGTs (Tables
 1 and 2). For BIT, only the Caribbean sample has different variances (Table 1).
 240 The slightly higher variance using `getTEX86` can be explained by the number of
 co-eluting peaks falling near the branched GDGTs, which occasionally led the
 program to identify peak boundaries inconsistently. Recent improvements in
 the chromatography of branched GDGTs, which are able to better separate the
 various isomers present (Becker et al., 2013; De Jonge et al., 2013), will result
 245 in more consistent integration. However, we note that the standard deviation
 of the BIT value for the Caribbean sample using `getTEX86` (0.004) is still low.

Figure 4 shows box plots of the $U_{37}^{K'}$, $U_{38_{et}}^K$, $U_{38_{me}}^K$, $U_{37}^{K'}$ total (concentration
 of C₃₇ alkenones), and U_{38}^K total (concentration of C₃₈ alkenones) values for
 the North Atlantic, subtropical Atlantic, and Gulf of Aden sediment samples.
 250 Table 3 shows the statistics for the $U_{37}^{K'}$, $U_{38_{et}}^K$, and $U_{38_{me}}^K$ values for manual and
 automated integration and Table 4 shows the statistics for $U_{37}^{K'}$ total and U_{38}^K
 total. The North Atlantic and Gulf of Aden samples have different means for
 manual and automated integration for $U_{37}^{K'}$, while the subtropical Atlantic has
 the same mean. The differences between the automated and manual means for
 255 the North Atlantic and the Gulf of Aden samples, while statistically significant,
 are very small (0.002) and on the order of analytical precision for duplicate
 analyses (0.002), suggesting no substantial differences between automated and
 manual integration.

We observe greater differences between the automated and manual results

260 for $U_{38_{et}}^K$ and $U_{38_{me}}^K$ (Table 3). This is because the C_{38} alkenones slightly co-elute with one another (c.f. Fig. 1), and the manual integration method will therefore underestimate peak areas. Manual integration will result in either underestimation or overestimation of the $U_{38_{et}}^K$ and $U_{38_{me}}^K$ values, depending on which ethyl/methyl pairs co-elute to the greatest degree. In our data, the
 265 $C_{38:2et}$ and $C_{38:2me}$ elute closest to one another, with an average separation of 0.18 minutes, compared to an average separation of 0.28 minutes for $C_{38:3et}$ and $C_{38:3me}$. This configuration tends to produce lower $U_{38_{et}}^K$ and $U_{38_{me}}^K$ values when using manual integration, as more of the $C_{38:2}$ peak areas are excluded. For example, the automated $U_{38_{et}}^K$ value for the North Atlantic sample is 0.009
 270 higher than the manual value due to differences in the $C_{38:2et}$ peak areas: this peak co-elutes with the $C_{38:2me}$ peak and another compound, and so a greater portion of its area is excluded during manual integration. For $U_{38_{me}}^K$, the automated mean values are higher for all of the samples (Table 3) due to differences in the $C_{38:2me}$ peak area, which is systematically underestimated by the manual
 275 method.

All samples have the same variance for manual and automated integration for $U_{37}^{K'}$ and $U_{38_{me}}^K$. For $U_{38_{et}}^K$, only the Gulf of Aden sample has different variances. In this case, the $C_{38:3et}$ peak is very small and co-elutes with another peak. Our algorithm occasionally has difficulty identifying the peak boundary,
 280 leading to high variance in the final $U_{38_{et}}^K$ values.

All samples have different means for both $U_{37}^{K'}$ total and U_{38}^K total (Table 4). For $U_{37}^{K'}$ total, although the differences are statistically significant, they are also small (0.02, 0.03, and 0.01 $\mu\text{g/g}$ sediment for the Subtropical Atlantic, North Atlantic, and Gulf of Aden samples, respectively). The differences are greater
 285 for U_{38}^K total. Because the $C_{38:2et}$ peaks often co-elute with the $C_{38:2me}$ peaks, and the $C_{38:3et}$ peaks often co-elute with the $C_{38:3me}$ peaks, a portion of these peaks are excluded from manual integration. This leads to consistently higher U_{38}^K total values for the automated integration. All samples have the same variance for $U_{37}^{K'}$ total and U_{38}^K total, indicating comparable precision between
 290 the automated and manual methods (Table 4).

To demonstrate the applicability and performance of ORIGAmI to GDGT and alkenone time series, we compared manual and automated results for TEX₈₆, BIT, and the three alkenone indices measured on two sediment cores, OCE 437-7 GC27 from the west African margin (subtropical Atlantic) and P178-15P from the Gulf of Aden. Figure 5 shows the TEX₈₆ and BIT time series from the Gulf of Aden core P178-15P. There is excellent agreement between the automated and manual TEX₈₆ values. Automated values are on average slightly lower, but are within analytical error. The automated and manual BIT values also show good agreement, and are well within analytical error of one another. As was the case with the three different sediment samples (Table 1, Fig. 3), the automated BIT values for P178-15P are generally higher, as ORIGAmI does not exclude areas where the target compounds co-elute with other peaks.

Figure 6 shows the $U_{37}^{K'}$, $U_{38_{et}}^K$, and $U_{38_{me}}^K$ time series from OCE 437-7 GC27. There is excellent agreement between the automated and manual values for all three indices, and the $U_{37}^{K'}$ and $U_{38_{et}}^K$ values are nearly indistinguishable. Larger differences are observed for $U_{38_{me}}^K$, where the automated values tend to be higher. As was the case with the different sediment samples (Table 3, Fig. 4) this difference is due to the underestimation of the C_{38:2} peak area by manual integration.

A recent manuscript by Dillon and Huang (2015) presented TEXPRESS, a software package which also analyzes GDGT LC-MS data. Like ORIGAmI, TEXPRESS performs better than manual integration methods when presented with co-eluting peaks. The underlying principles behind both packages are similar, only TEXPRESS fits exponentially modified Gaussian curves rather than standard Gaussians. We chose a standard Gaussian approach because the computation was faster and generally, most GC and HPLC peaks are standard Gaussians. The exponential Gaussian approach of TEXPRESS, however, offers an advantage for calculating areas of asymmetric peaks that might arise from column overloading or close co-elution of two peaks. Arguably, however, peaks of this nature should be fixed by improving the chromatography.

Aside from the Gaussian fits, the main differences between the two packages

are 1) their user interface and 2) the flexibility of the code. Regarding user interface, TEXPRESS only allows users to analyze one data file at a time and utilizes a GUI. In contrast, ORIGAmI is run by a single command line, and
 325 can process many data files at once. Therefore, ORIGAmI is more efficient at processing the large number of files typically associated with paleoclimate time series. Regarding the code, TEXPRESS was designed specifically for GDGTs. ORIGAmI was designed to be used for any kind of GC or HPLC peak picking application that users might require. While we illustrated only the specific
 330 applications to TEX_{86} and $\text{U}_{37}^{K'}$ in this manuscript, the ORIGAmI package also includes the functions `getGCAreas` and `getHPLCAreas` that can be used to pick peaks for any type of compound. Therefore, ORIGAmI represents a more general approach to the problem of automated peak detection.

4. Conclusions

335 ORIGAmI is a flexible software package designed to analyze sediment samples quickly and accurately. Our comparison of manual and automated integration across different sample types demonstrates that in most cases, ORIGAmI produces similar mean values and variances for TEX_{86} , BIT, the alkenone indices, and lipid concentrations. Differences primary emerge where there are
 340 co-eluting peaks, and manual integration excludes a portion of the peak area. In such cases, ORIGAmI will provide a more accurate estimate of the target index. This may be especially advantageous for the analysis of $\text{U}_{38_{me}}^K$. $\text{U}_{38_{me}}^K$ has a higher SST tolerance than $\text{U}_{37}^{K'}$, and therefore may improve alkenone-based SST estimation in the tropics, but has generally not been used due to the difficulty
 345 of manually integrating the methyl and ethyl C38 ketones (Herbert, 2003).

Although in most cases ORIGAmI fits the data well, because it fits Gaussian curves it will be less accurate for very asymmetrical peaks. For alkenone samples with high concentrations, peaks are sometimes asymmetrical, and manual integration may still be necessary for such samples. In addition, although
 350 the precision of ORIGAmI is generally comparable to manual integration, when

co-eluting peaks elute so closely that the boundary between peaks is difficult to determine, ORIGAmI may not choose peak boundaries consistently, resulting in higher variance. In both cases, there is no substitute for better chromatography.

Within the ORIGAmI package, the principles of `getTEX86` and `getAlkenones` are generalized in the functions `getGCAreas` and `getHPLCAreas`, allowing the user to automate integration of a set of target peaks of their choosing. Although not specifically demonstrated here, these functions are based on identical algorithms and are subject to the same limitations as `getAlkenones` and `getTEX86`. Together, the four functions of ORIGAmI enable rapid, accurate, and precise analysis of GC and HPLC data for paleoclimatic reconstruction.

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TEX₈₆						
	Arctic		North Atlantic		Caribbean	
	Auto	Manual	Auto	Manual	Auto	Manual
Mean	0.408	0.405	0.544	0.542	0.682	0.686
Median	0.408	0.404	0.543	0.542	0.683	0.686
Standard Dev.	0.004	0.004	0.006	0.004	0.003	0.002
t Test	0		0		1	
F Test	0		0		0	
Mean SST (°C)	6.03	5.89	18.99	18.88	25.61	25.84
Median SST (°C)	6.05	5.85	18.95	18.89	25.64	25.86
Standard Dev. (°C)	0.261	0.239	0.228	0.256	0.150	0.144

BIT						
	Arctic		North Atlantic		Caribbean	
	Auto	Manual	Auto	Manual	Auto	Manual
Mean	0.297	0.292	0.078	0.077	0.183	0.176
Median	0.296	0.292	0.079	0.077	0.184	0.176
Standard Dev.	0.006	0.005	0.002	0.001	0.004	0.002
t Test	1		1		1	
F Test	0		0		1	

Table 1: TEX₈₆ and BIT values for the Arctic, North Atlantic, and Caribbean samples, along with the results of the Welch’s t-test and the F test for equality of variances. For the t-test, 1 indicates that you can reject the null hypothesis that the two samples have equal means ($p < 0.05$), and 0 indicates that you cannot reject the null hypothesis. For the F Test, 1 indicates that the null hypothesis that the two samples have equal variance can be rejected at $p < 0.05$, while 0 indicates that the null hypothesis cannot be rejected. Sea-surface temperatures (SSTs) were calculated using the BAYSPAR calibration (Tierney and Tingley, 2014).

Concentration of isoGDGTs (ng/g sediment)				
	Arctic		North Atlantic	
	Auto	Manual	Auto	Manual
Mean	26.2	24.9	99.6	95.2
Median	25.8	24.6	99.7	95.2
RSD (%)	5.3	4.8	1.4	1.4
t Test	1		1	
F Test	0		0	

Concentration of brGDGTs (ng/g sediment)				
	Arctic		North Atlantic	
	Auto	Manual	Auto	Manual
Mean	28.3	27.6	25.1	24.5
Median	28.1	27.5	25.2	24.5
RSD (%)	5.2	4.8	2.1	1.8
t Test	0		1	
F Test	0		0	

Table 2: Concentrations of isoGDGTs and brGDGTs (ng/g sediment) for the Arctic and North Atlantic samples, along with the results of the Welch's t-test and the F test for equality of variances. RSD denotes the relative standard deviation; t-test and F test results are presented as described in the caption of Table 1.

U ^{K'} ₃₇						
	Subtropical Atlantic		North Atlantic		Gulf of Aden	
	Auto	Manual	Auto	Manual	Auto	Manual
Mean	0.573	0.572	0.256	0.254	0.907	0.905
Median	0.570	0.568	0.255	0.253	0.906	0.904
Standard Dev.	0.008	0.008	0.002	0.002	0.001	0.001
t Test	0		1		1	
F Test	0		0		0	
Mean SST (°C)	16.03	16.00	6.40	6.37	26.12	26.08
Median SST (°C)	15.92	15.88	6.38	6.33	26.12	26.06
Standard Dev. (°C)	0.251	0.252	0.070	0.069	0.021	0.025
U ^K _{38_{et}}						
	Subtropical Atlantic		North Atlantic		Gulf of Aden	
	Auto	Manual	Auto	Manual	Auto	Manual
Mean	0.631	0.636	0.361	0.352	0.956	0.951
Median	0.628	0.634	0.360	0.352	0.958	0.952
Standard Dev.	0.007	0.007	0.003	0.003	0.008	0.001
t Test	1		1		1	
F Test	0		0		1	
U ^K _{38_{me}}						
	Subtropical Atlantic		North Atlantic		Gulf of Aden	
	Auto	Manual	Auto	Manual	Auto	Manual
Mean	0.450	0.438	0.189	0.184	0.855	0.844
Median	0.447	0.435	0.189	0.185	0.856	0.843
Standard Dev.	0.008	0.008	0.002	0.002	0.002	0.003
t Test	1		1		1	
F Test	0		0		0	

Table 3: $U_{37}^{K'}$, U_{38et}^K , and U_{38me}^K values for the Subtropical Atlantic, North Atlantic, and Gulf of Aden samples, along with the results of the Welch's t-test and the F test for equality of variances; t-test and F test results are presented as described in the caption of Table 1. SSTs were calculated using the calibration of [Müller et al. \(1998\)](#).

$U_{37}^{K'}$ Total ($\mu\text{g/g}$ sediment)						
	Subtropical Atlantic		North Atlantic		Gulf of Aden	
	Auto	Manual	Auto	Manual	Auto	Manual
Mean	0.95	0.93	2.10	2.07	1.26	1.25
Median	0.94	0.93	2.10	2.07	1.26	1.25
RSD (%)	2.0	2.2	0.5	0.7	0.9	0.8
t Test	1		1		1	
F Test	0		0		0	

U_{38}^K Total ($\mu\text{g/g}$ sediment)						
	Subtropical Atlantic		North Atlantic		Gulf of Aden	
	Auto	Manual	Auto	Manual	Auto	Manual
Mean	1.05	1.01	2.66	2.57	1.29	1.18
Median	1.04	1.00	2.66	2.56	1.29	1.18
RSD (%)	1.9	2.1	0.5	0.7	1.7	1.2
t Test	1		1		1	
F Test	0		0		0	

Table 4: $U_{37}^{K'}$ Total and U_{38}^K Total ($\mu\text{g/g}$ sediment) for the Subtropical Atlantic, North Atlantic, and Gulf of Aden samples, along with the results of the Welch's t-test and the F test for equality of variances. RSD denotes the relative standard deviation; t-test and F test results are presented as described in the caption of Table 1.

Figure 1: A sample GC trace containing alkenones with integration by `getAlkenones`. The blue lines show the parts of the trace that were fit to a Gaussian function. Note that the algorithm uses only the descending side of the C37:3 compound to fit the peak, as it co-elutes with another compound. The resulting Gaussian peak areas are shown in gray. The Gaussian peaks of C38:2_{et} C38:2_{me} overlap but neither tail is cut off. The values in the upper right hand corner show the calculated $U_{37}^{K'}$ for the automated (Auto) vs. manual (Hand) methods.

Figure 2: A sample HPLC trace containing GDGTs with integration by `getTEX86`. The thick black line is the total ion current (TIC), while the thin black lines are the extracted ion chromatograms (EIC) of individual ions. GDGT peak areas are traditionally integrated in the EICs (Schouten et al., 2007b), the areas of which differ slightly from the TIC for some of the compounds. The blue lines are the part of the trace that were fit to a Gaussian curve. The values in the upper center area show the calculated TEX_{86} for the automated (Auto) vs. manual (Hand) methods.

Figure 3: TEX₈₆ and BIT values for automated and manual integration for the Arctic, Caribbean, and North Atlantic sediment samples, and concentrations of isoGDGTs and brGDGTs (ng/g sediment) for the Arctic and North Atlantic samples. If q_1 and q_3 are the first and third quartiles of the data, respectively, then the whiskers on the box plots are $q_3 + 1.5(q_3 - q_1)$ and $q_1 - 1.5(q_3 - q_1)$. Any data above or below these whiskers are considered outliers.

Figure 4: $U_{37}^{K'}$, U_{38et}^K , U_{38me}^K , $U_{37}^{K'}$ total ($\mu\text{g/g}$ sediment), and U_{38}^K total ($\mu\text{g/g}$ sediment) values for automated and manual integration for the Subtropical Atlantic, North Atlantic, and Gulf of Aden sediment samples. If q_1 and q_3 are the first and third quartiles of the data, respectively, then the whiskers on the box plots are $q_3 + 1.5(q_3 - q_1)$ and $q_1 - 1.5(q_3 - q_1)$. Any data above or below these whiskers are considered outliers.

Figure 5: TEX_{86} and BIT time series of sediment samples from core P178-15P from the Gulf of Aden. Grey error bars denote the 1σ analytical error, 0.004. Sea-surface temperatures (SSTs) for the upper plot were calculated using the BAYSPAR calibration (Tierney and Tingley, 2014).

Figure 6: $U_{37}^{K'}$, $U_{38_{et}}^K$, and $U_{38_{me}}^K$ time series of sediment samples from core OCE437-7 GC27 from the subtropical Atlantic. SSTs for the upper panel were calculated using the calibration of Müller et al. (1998). Grey error bars denote the 1σ analytical error, 0.004. However, the analytical error is very small compared to the scale of the y -axis, and therefore almost impossible to see.