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Analytical Considerations for the Use of the Paleothermometer Tetraether Index₈₆ and the Branched vs Isoprenoid Tetraether Index Regarding the Choice of Cleanup and Instrumental Conditions

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The tetraether index of tetraethers consisting of 86 carbons (TEX₈₆) is a novel proxy applied to obtain paleotemperature reconstructions from marine and lacustrine settings. It is usually applied alongside the branched vs isoprenoid tetraether (BIT) index, which provides paleoenvironmental information as well as information on the reliability of TEX₈₆. Both indices are calculated via the analysis of glycerol dialkyl glycerol tetraethers or GDGTs by means of high-performance liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry (HPLC/APCI-MS). Here we test the performance of alternative methods for sample cleanup and instrumental analysis. In particular, we evaluate using alkaline hydrolysis as an alternative cleanup step to alumina column fractionation and show that the resulting TEX₈₆ and BIT are statistically equivalent. We also test two different adsorbents in the activated or deactivated state for preparative column fractionation and show that any of them can be used to measure TEX₈₆ but that a certain discrimination between GDGTs used in the BIT index can occur. Regarding the mass spectrometer design, an ion-trap is shown to be as precise as a quadrupole mass spectrometer for GDGT analysis. Some differences are observed for TEX₈₆ and especially for BIT values obtained from both MS designs. We provide evidence that the APCI conditions are at least partly responsible for these differences. We recommend caution when comparing BIT values among laboratories as this index seems to be especially sensitive to analytical conditions.

Quantitative reconstruction of past sea surface temperature (SST) is of primary importance to understand the mechanisms responsible for natural climate variability. Geochemical proxies developed to estimate past SST based on organic molecules or

trace elements have proven invaluable for such a purpose.^{1–3} One of the most novel approaches is based on the relative concentrations of membrane lipids synthesized by aquatic Archaea, the glycerol dialkyl glycerol tetraethers or GDGTs⁴ (Figure 1, structures 0–4'). These lipids with an isoprenoidal structure are ubiquitous in the marine water column and eventually accumulate in the ocean floor sediments. Their internal cyclization has been found to be mainly determined by water temperature,^{5,6} which has been expressed as the TEX₈₆ (tetraether index of tetraethers consisting of 86 carbons) to derive a proxy to estimate past SST;⁷

$$\text{TEX}_{86} = \frac{[\text{GDGT-2}] + [\text{GDGT-3}] + [\text{GDGT-4'}]}{[\text{GDGT-1}] + [\text{GDGT-2}] + [\text{GDGT-3}] + [\text{GDGT-4'}]} \quad (1)$$

Its calibration to derive quantitative past temperature reconstructions has been undertaken using a suite of 223 core-top sediments with a worldwide distribution, resulting in the following equation:⁸

$$\text{SST} = -10.78 + 56.2(\text{TEX}_{86}) \quad (2)$$

TEX₈₆ has been applied to reconstruct SST in diverse oceanic environments.^{9–12} A remarkable advantage of TEX₈₆ over other

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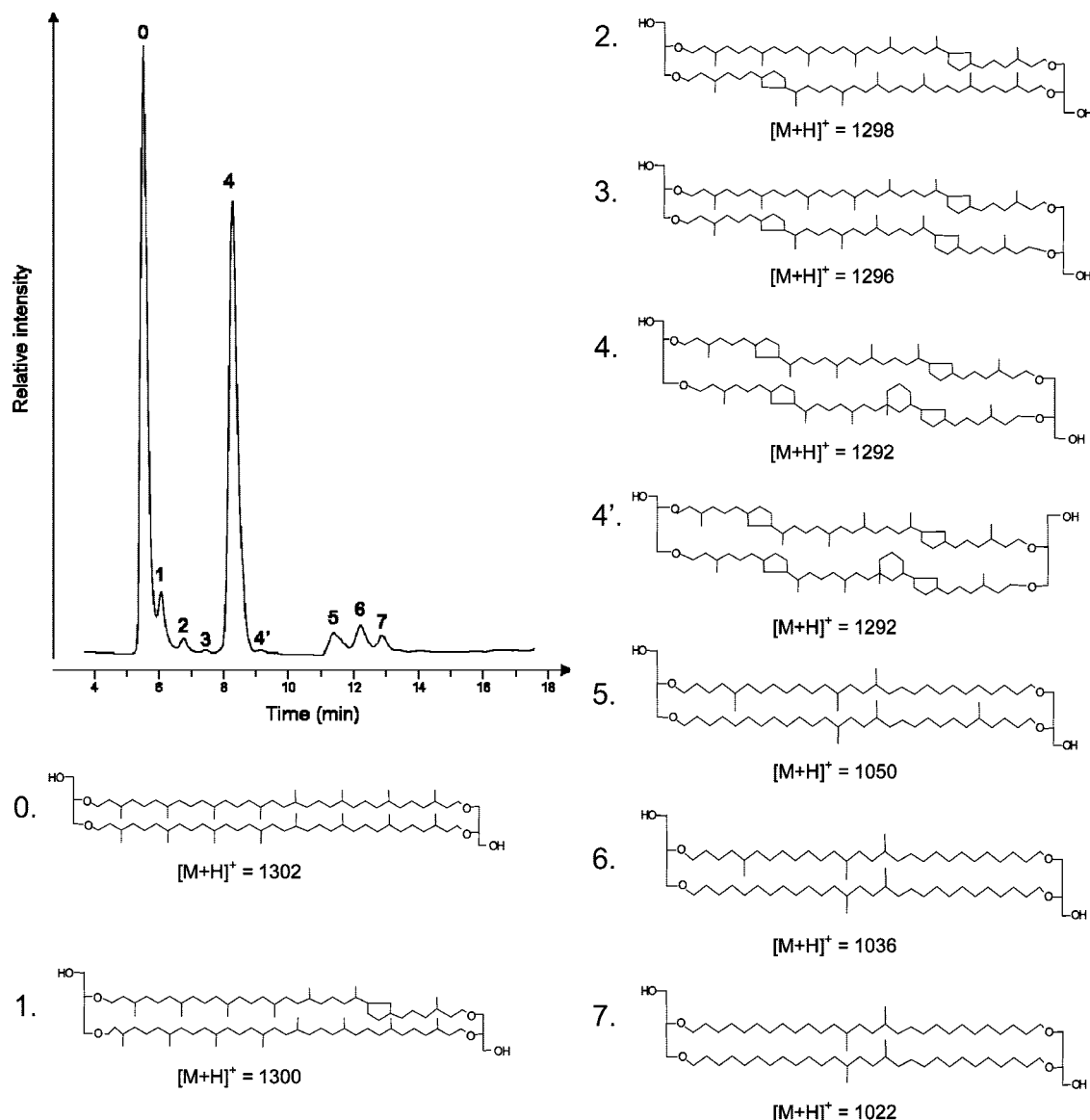


Figure 1. Base peak chromatogram and structures of glycerol dialkyl glycerol tetraethers or GDGTs and the corresponding masses of the protonated molecules. Note that structure 4' is a regioisomer of structure 4 (crenarchaeol).

proxies is that it can also be applied to lake sediments to reconstruct past surface lake water temperature, albeit with a different calibration equation than in eq 2.^{13–15} In fact, TEX₈₆ is the only method available to paleoclimatologists to quantify past surface water temperatures in lakes. Because of the thermal stability of the GDGTs, TEX₈₆ can be applied in marine environments beyond the temporal range of most conventional proxies.

Another group of GDGTs which are ubiquitous in aquatic sediments have the characteristic feature that they present

branched instead of isoprenoid structures (Figure 1, structures 5–7). The branched GDGTs have a terrestrial origin,¹⁶ and in paleo-studies their presence in aquatic settings is used to assess the relative changes in fluvial or runoff input of soil organic matter, which can be used to gain information on the past hydrological cycle. This information is derived from the branched vs isoprenoid tetraether (BIT) index¹⁷ (eq 3), which quantifies the relative abundance of the soil-originated branched GDGTs versus the isoprenoid crenarchaeol (GDGT-4), which is predominantly derived from aquatic Archaea.

$$\text{BIT} = \frac{[\text{GDGT-5}] + [\text{GDGT-6}] + [\text{GDGT-7}]}{[\text{GDGT-4}] + [\text{GDGT-5}] + [\text{GDGT-6}] + [\text{GDGT-7}]} \quad (3)$$

Thus, typical values for BIT in soils are around 0.90, while open marine sediments display values close to 0.01, and coastal

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sediments usually have BIT values in between those two extremes.^{17–20} This index is used to assess the reliability of TEX₈₆ as this is compromised as a SST proxy if the sediment sample contains significant amounts of terrestrial GDGTs.²¹ The value of the BIT index as a climate proxy has also been demonstrated in several recent multiproxy studies focusing on the abundance and distribution of terrestrial organic matter in continental margins,^{18,19,22–24} for instance tracing the reactivation of the European hydrological system at the onset of the last deglaciation.²⁵ Consequently, as both TEX₈₆ and BIT are being increasingly used worldwide as climate proxies, there is much interest to clarify any constraints regarding their measurement. Given their novelty, very few reports address the robustness of the analytical methodology employed to quantify the relative abundance of GDGTs in environmental samples.

The most common analytical method used to measure both GDGTs indices in a sediment sample includes the organic solvent extraction of the organic matter, followed by a fractionation of the extract in apolar and polar fractions with activated aluminum oxide (alumina).^{26,27} The polar fraction containing the GDGTs is then analyzed by high-performance liquid chromatography–mass spectrometry (HPLC–MS) with an atmospheric pressure chemical ionization interface (APCI) and a quadrupole detector.^{26,27} Several methods for the extraction of the organic matter from sediment samples were compared in a recent paper by Schouten et al.²⁷ The authors showed that there was no significant difference between Soxhlet, ultrasonic, and accelerated solvent extraction methods. They also compared two analytical columns and two mass spectrometers with quadrupole design and evaluated the advantages of full scan vs single ion monitoring (SIM) mode. In this study, the reproducibility for the TEX₈₆ index was ± 0.004 , which translates into a temperature uncertainty of ± 0.3 °C.²⁷

The aim here is to discuss the comparability of alternative analytical methods to measure the TEX₈₆ and BIT, using procedures and equipment which are not employed commonly to measure the GDGT proxies but are nonetheless frequently used by organic geochemists. For instance, it might be desirable in some cases to remove certain compounds in samples with a rich organic content in order to facilitate the detection of compounds such as the GDGTs. For this purpose, we tested alkaline hydrolysis (saponification) as an alternative

option to column fractionation for the cleanup of organic extracts. We also tested different adsorbents and their degree of activation for the fractionation of the extracts. In addition we explored different analytical instrumental configurations, in particular, to measure TEX₈₆ and BIT with an ion trap mass spectrometer as an alternative to using a quadrupole mass spectrometer.

EXPERIMENTAL SECTION

Sediment Extraction. A set of 23 marine and lacustrine sediments, including both organic-rich ocean sediment surface and below-surface sediment samples were freeze-dried and homogenized by mortar and pestle. Between 1–2 g of the freeze-dried sediments were extracted by microwave assisted extraction (MAE) using a mixture of dichloromethane (DCM)/methanol (3:1, v/v).²⁸ All extracts were dried by vacuum rotary evaporation, and half of each extract was subject to alkaline hydrolysis whereas the other half was fractionated with preparative column chromatography.

Additionally, approximately 15 g of freeze-dried sediment from Lake Banyoles (Catalonia, Spain) were ground before ultrasonic extraction with a mixture of DCM/methanol (3:1, v/v). This extract was split in several aliquots to test different adsorbents in preparative column chromatography.

Comparison of Cleanup Procedures. For alkaline hydrolysis, the extracts were redissolved in 1.5 mL of methanol and 3 mL of 8% potassium hydroxide in methanol and left overnight. The samples were concentrated to 1 mL by vacuum rotary evaporation, and the neutral compounds were recovered by liquid extraction with hexane (3 × 3 mL).

To assess the recovery of GDGTs when water is used to remove remaining salts from the hexane fraction, eight aliquots of a GDGT-0 standard solution were saponified and extracted with hexane, and four of them were further extracted with water (2 × 1 mL). A nonsaponified aliquot was used as a control.

For preparative column chromatography, glass Pasteur pipets were filled with activated alumina (Merck; placed for a minimum of 5 h at 450 °C) and the extracts were eluted with 3 mL of a mixture of hexane/DCM (9:1, v/v) and 3 mL of DCM/methanol (1:1, v/v). The last polar fraction contained the GDGTs.

Previous to the injection in the HPLC–MS, all extracts were evaporated, redissolved in hexane/*n*-propanol (99:1, v/v), and filtered through 0.45 μ m PVDF filters.

Comparison of Adsorbents in Preparative Column Chromatography. Aliquots of Banyoles extract were fractionated with glass Pasteur pipets filled with the different adsorbents. These were activated alumina (placed for a minimum of 5 h at 450 °C), activated silica (up to 5 h at 120 °C), and their deactivated homologues (5% dry weight with Milli-Q water, thoroughly mixed for 20 min and stored in a desiccator overnight). In the alumina columns, the extracts were eluted with 3 mL of solvent, whereas for silica the elution volume was 4 mL, the apolar and polar solvents being the mixtures indicated above for column chromatography. Triplicates of the experiments and a blank control were performed. The polar fraction was evaporated and redissolved in hexane/*n*-propanol (99:1, v/v) and filtered through 0.45 μ m PVDF filters. An analysis of variance was undertaken with the results using the SPSS statistical software.

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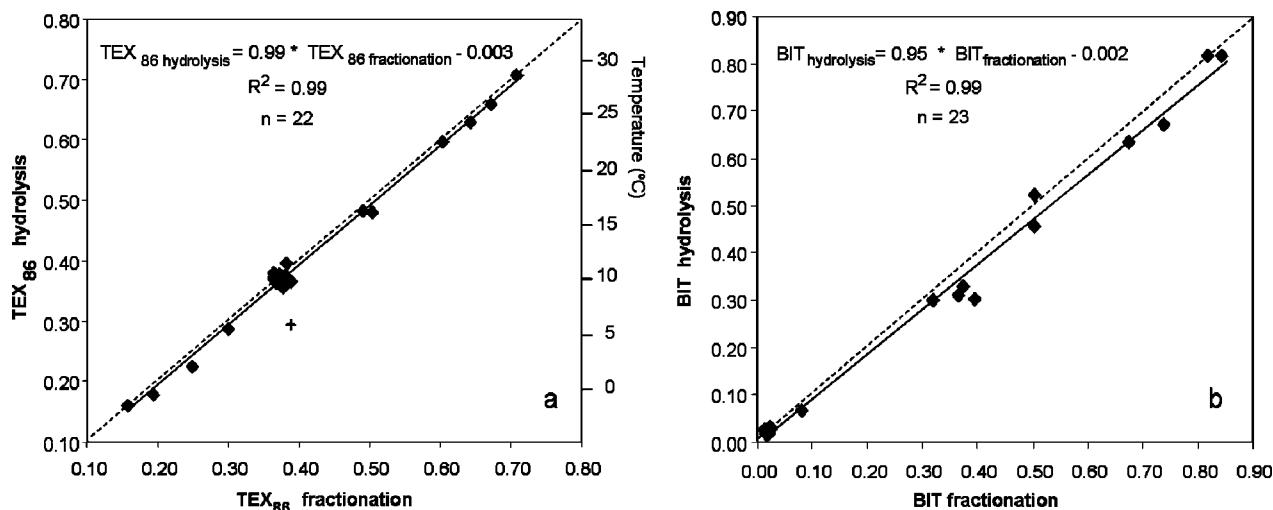


Figure 2. Cross-plots of TEX₈₆ (a) and BIT values (b) obtained with the two cleanup methods tested: activated alumina column fractionation and alkaline hydrolysis (saponification). The cross symbol indicates an outlier data point. The standard deviation of the injections is not represented as it is smaller than the dot sizes. The dashed line indicates the 1:1 correspondence and is drawn only for illustration purposes.

HPLC–APCI–MS Systems. The equipment used included a Dionex P680 HPLC system coupled to a Thermo Finnigan TSQ Quantum Discovery Max quadrupole mass spectrometer with an APCI interface, hereafter referred to as the quadrupole system. The APCI was used in the positive mode, and the main parameters were optimized by infusion of a GDGT-0 solution (kindly provided by Dr. H. Morii, UOEH, Kitakyushu, Japan) and set as follows: vaporizer temperature 400 °C, corona discharge 3 μ A, sheath gas pressure 6.5 Pa, auxiliary gas (N₂) pressure 0.7 Pa, and capillary temperature 200 °C. GDGTs were detected in SIM mode and quantified by the integration of their protonated molecule ($[M + H]^+$, see Figure 1). A second system, composed of an Agilent HPLC 1100 series coupled to a Bruker 3000 ion-trap mass spectrometer with an APCI interface, hereafter referred to as the ion-trap system, was also used for comparison. The positive mode in the APCI interface coupled to the ion-trap was set with the following parameters: vaporizer temperature 300 °C, dry temperature 250 °C, corona discharge 5 kV, capillary voltage 4200 V, nebulizer gas pressure 60 psi, and dry gas flow 6 L/min. Two m/z ranges were recorded to include the BIT and TEX₈₆ GDGTs, namely, m/z 1010–1060 and m/z 1285–1310, respectively. The integration of the relevant GDGTs was done on the individual extracted chromatograms of $[M + H]^+ \pm 0.5$ m/z units. The TEX₈₆ and BIT indices were calculated using eqs 1 and 3, and TEX₈₆ was converted to temperature values using eq 2.

HPLC Columns and Conditions. The samples were eluted in a Prevail CN column (150 mm \times 2.1 mm, 3 μ m; Alltech) starting with an isocratic mixture of 98.5% hexane and 1.5% *n*-propanol for 4 min; afterward, the *n*-propanol was increased to 5% in 11 min and to 10% in 1 min; this proportion was held for 4 min and followed by a return to 1.5% *n*-propanol in 1 min and a stabilization period of 9 min. The solvent program is derived and modified from Schouten et al.²⁷ The flow rate was 0.3 mL/min, and the injection volume was 10 μ L. The analysis of a large number of samples in paleoclimatology studies requires detailed consideration on cost and waste management issues during the analysis, and in particular for the GDGTs analysis, the volume of organic solvents used in the HPLC is a relevant concern. We tested an HPLC flow reduction with the aim to observe the change impinged

on the stability of the GDGTs indices, given the resulting change in GDGTs peak shape and resolution. Alongside flow reduction, we used a smaller-particle size column to keep a comparable head pressure in the HPLC system. Thus, the Tracer Nucleosil CN column (4 mm \times 150 mm, 5 μ m; Teknokroma), which had been used in our laboratory for previous analysis at a flow rate of 1 mL/min, was compared to the aforementioned Prevail CN column (150 mm \times 2.1 mm, 3 μ m; Alltech) at a flow rate of 0.3 mL/min. The elution program with the Tracer Nucleosil CN column started with an isocratic mixture at 99% hexane and 1% *n*-propanol; after 12 min, *n*-propanol was increased to 10% in 1 min and these conditions were held for 3 min; *n*-propanol was returned to 1% in 1 min and held until the end of the run at 22 min. The flow rate was 1 mL/min and only increased to 2 mL/min between 13–16 min for column cleaning. This flow reduction and column comparison was tested on the ion-trap system.

Comparison of Mass Spectrometers. To investigate the reproducibility of the analysis using different designs of mass spectrometer, we compared the TEX₈₆ and BIT values for 22 samples obtained with the ion-trap and the quadrupole spectrometers using the Prevail CN column and respective APCI interfaces with the aforementioned parameters.

RESULTS AND DISCUSSION

Cleanup Methods: Saponification vs Alumina Fractionation. A set of sediment extracts were subjected to two different cleanup methods, alkaline hydrolysis and fractionation through activated alumina columns, and their GDGT distribution was analyzed by means of an HPLC–APCI–quadrupole MS. The resulting TEX₈₆ and BIT values obtained with the two procedures are plotted in Figure 2, which shows no sign of systematic difference between the two methods. We identified and discarded outliers for TEX₈₆ if any point fell beyond 3 standard deviations of the linear regression between saponified vs fractionated extracts (see Figure 2). A paired *t* test of the remaining 22 TEX₈₆ values indicated that the alkaline hydrolysis and alumina fractionation cleanup methods did not result in significantly different TEX₈₆ values (*t* critical value = 2.83, *t* empirical value = 2.41, *P* = 0.01). The standard deviation of the regression

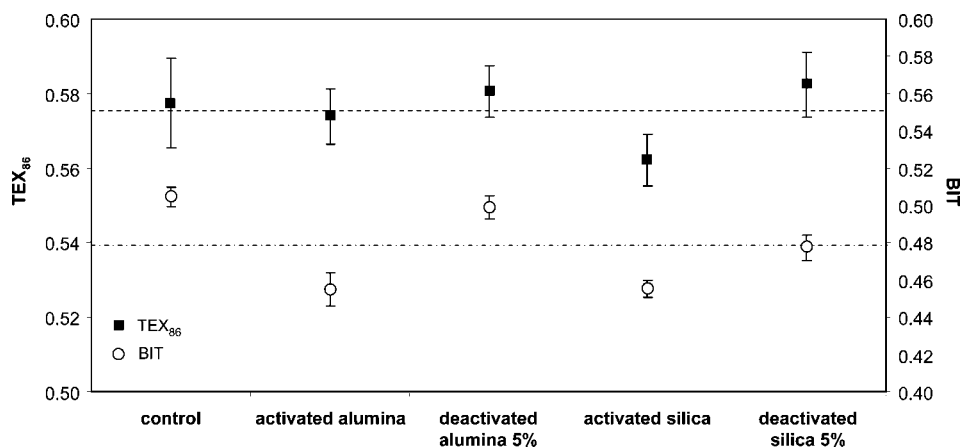


Figure 3. Comparison of the effect of the adsorbent type in column fractionation on TEX₈₆ and BIT values for a sediment from Lake Banyoles. Error bars indicate $\pm 1\sigma$ ($n = 3$), and the dashed lines show the average index value.

including the 22 values is 0.013 or ~ 0.7 °C. This was half the precision we routinely obtain with the HPLC–quadrupole-MS, 1σ being 0.006 or ~ 0.3 °C. Regarding the BIT, a paired t test based on the 23 values also points at a nonsignificant difference between alkaline hydrolysis and alumina fractionation (t critical value = 2.82, t empirical value = 2.50, $P = 0.01$). Therefore, both methods are in principle comparable in terms of TEX₈₆ and BIT measurement in lake and ocean sediment samples with different contents of organic matter. However, the saponification step may have a critical drawback if in the extraction process after saponification water is used to remove excess salt in the hexane extracts. We used a standard solution of GDGT-0 to calculate recoveries of the procedure. When the water extraction step was removed, the recovery of GDGT-0 of four replicate analyses was on average 104.6% ($\sigma = 4.5\%$, $n = 4$), which dropped to 6.1% ($\sigma = 4.1\%$, $n = 4$) when water extraction after hydrolysis was undertaken. This could lead to a massive underestimation of the amounts of GDGTs in the samples and clearly should be avoided.

Cleanup Methods: Comparison of Adsorbents. Four different adsorbents were tested in preparative chromatography. The resulting TEX₈₆ and BIT values obtained from triplicate analyses are compared in Figure 3. We confirmed the normal distribution of the data sets with a Kolmogorov–Smirnov test ($P = 0.925$ for TEX₈₆ and $P = 0.765$ for BIT) and the homogeneity of variance ($P = 0.600$ for TEX₈₆ and $P = 0.626$ for BIT) previous to the analysis of variance (one-way ANOVA test). According to the ANOVA, the five methods tested (four adsorbents and a control) yielded significantly different results in terms of BIT ($P < 0.001$) but not for TEX₈₆ ($P = 0.105$). There was a maximum TEX₈₆ difference between methods of 0.020 or 1.1 °C (activated silica vs deactivated silica) and a minimum difference of 0.002 or 0.1 °C (deactivated silica vs deactivated alumina). Thus, it seems that the fractionation with the compared adsorbents is not discriminating between the isoprenoidal GDGTs related to TEX₈₆. On the other hand, the maximum difference between the average BIT values of the tested methods is 0.050 (control vs activated alumina and silica). It can be argued that for the qualitative nature of the proxy and its range of possible values (0–1) this difference raises no restriction for the selection of the cleanup method.

However, results shown in Figure 3 suggest that the values of BIT in the hitherto published studies^{17,18,21,22} might contain a certain degree of bias if activated alumina was used for the cleanup of samples, and this hampers the comparability of the published results.

HPLC Column Effects. The indices TEX₈₆ and BIT were calculated for a set of 19 samples injected in two different HPLC columns operated at 0.3 and 1 mL/min. The columns had the same stationary phase but were different in terms of particle size, column diameter, and manufacturer. Given that the efficiency of a column is inversely proportional to the squared diameter and to the particle size, theoretically this gives a maximum increase of efficiency of ~ 6 times in changing columns from the Tracer Nucleosil CN to the Prevail CN. As the root square of column efficiency is proportional to the resolution it can provide, theoretically the resolution could be increased more than 2-fold with the Prevail CN in comparison to the Tracer CN tested. However this is compensated by the flow reduction, which was the final objective of the experiment. Column head pressure was comparable for both columns: in the Tracer Nucleosil CN column at 1 mL/min it was 59 bar, while a similar pressure (56 bar) was reached with the Prevail CN at 0.3 mL/min. The tested chromatographies did not produce significantly different TEX₈₆ results (paired t test; $n = 19$, t critical value = 2.88, t empirical value = 1.43, $P = 0.01$). However, there is a certain degree of dispersion of the TEX₈₆ results, indicated by a standard deviation of Tracer Nucleosil CN over Prevail CN results of 0.041 equivalent to 2.1 °C. Some samples show important differences in TEX₈₆ values calculated with the two HPLC columns (six samples show a difference larger than 1.7 °C, which is the current error of the TEX₈₆ calibration with SST[®]). At present, we have no explanation for the cause of the divergent TEX₈₆ values with the two columns, but generally, the high correlation between both sets of results allows the intercomparability of the TEX₈₆ values. The tested columns did also not significantly produce different BIT values (paired t test; $n = 18$, t critical value = 2.90, t empirical value = 1.92, $P = 0.01$), and only one sample shows a difference larger than 0.10. An important advantage of the Prevail CN column is that the narrower and smaller particle size column requires about 3 times less solvent volume compared to the

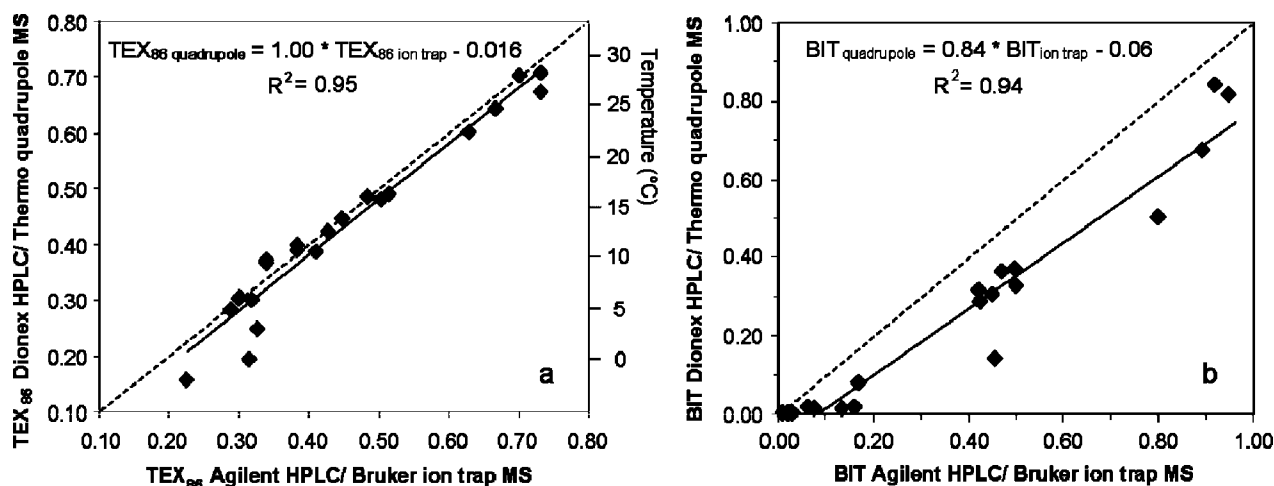


Figure 4. Cross-plots of TEX_{86} and BIT values obtained using the Dionex HPLC–Thermo quadrupole MS and the Agilent HPLC–Bruker ion-trap MS. The standard deviation of the injections is not represented as it is smaller than the dot sizes. The dashed line indicates the 1:1 correspondence and is drawn only for illustration purposes.

Trace CN, a significant cost and environmental advantage even if the run is lengthened by 7 min.

Comparison of Mass Spectrometers. With the aim of verifying whether BIT and TEX_{86} values obtained with the ion-trap MS are comparable to and as precise as the ones obtained with the quadrupole MS, a set of 22 sample extracts were injected in both systems using the same chromatographic conditions. The ion-trap system yielded a standard deviation of TEX_{86} from a triplicate injection of 0.007 or $\sim 0.4^\circ\text{C}$, while for the quadrupole system the standard deviation was 0.006 or $\sim 0.3^\circ\text{C}$. The BIT values from the same injections yielded a standard deviation of 0.002 for the ion-trap and 0.009 for the quadrupole system. Thus, no apparent difference in precision was obtained using both systems.

The cross-plot for TEX_{86} values (Figure 4a) suggests little systematic bias between the two HPLC–MS systems tested. The differences in TEX_{86} values for 18 out of the 22 samples range between 0.1 and 1.7°C . However, four samples show differences of $>3^\circ\text{C}$. They have in common that the GDGT-2 was slightly overestimated, and the GDGT-1 slightly underestimated in the ion-trap MS compared to the quadrupole MS. Furthermore, three of these samples belong to the lower range of the TEX_{86} index, below 0.3. However, at present the cause of this difference is not clear to us. A systematic difference between both systems is clearly observed for BIT, as the quadrupole yielded lower values than the ion-trap MS (Figure 4b). This could be related to the different analytical conditions employed in the MS system and a different response or ionization efficiency of isoprenoidal vs branched GDGTs, given that conditions in both systems were optimized with the isoprenoidal GDGT-0 but not with a branched GDGT.

To further appraise the effects of the MS conditions on the TEX_{86} and BIT, we investigated the effect on GDGT yields of changes in the corona current, the temperatures involved in the vaporization of the sample (vaporizer temperature), and the ion transfer to the vacuum region in the detector (capillary temperature). While the tested corona intensities ($3\text{--}15\ \mu\text{A}$) did not significantly change the relative GDGT yields, a larger

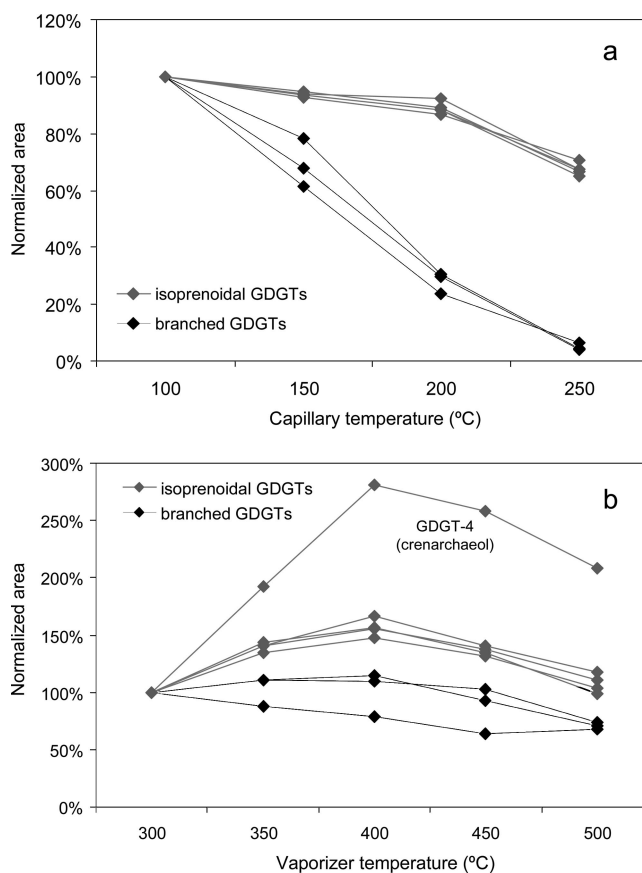


Figure 5. Effect of vaporizer temperature and capillary temperature on the branched (GDGT-5–7) and isoprenoidal (GDGT-1–4) GDGTs yields. Data points reflect only one injection and were obtained with a Dionex HPLC coupled to a Thermo Finnigan TSQ Quantum Discovery Max mass spectrometer via an APCI interface.

effect was observed for the temperatures (Figure 5). The ionization and transfer efficiency as derived from the peak areas differ between isoprenoidal GDGTs and branched GDGTs at different vaporizer temperatures ($300\text{--}500^\circ\text{C}$; Figure 5b), and even more at different capillary temperatures ($100\text{--}250^\circ\text{C}$; Figure 5a). This raises the question of how the indices TEX_{86} and BIT are affected by the optimization or tuning of the APCI. We

observed a maximum difference in TEX_{86} of 0.05 or $\sim 2.5\text{ }^{\circ}\text{C}$ (result from a single analysis). However, the BIT value of a sample rose from 0.15 to 0.45 when the capillary temperature was lowered from 200 to 100 $^{\circ}\text{C}$. The BIT values are more sensitive to the APCI conditions than TEX_{86} , as the BIT index is based on measuring two types of GDGTs, i.e., the branched and isoprenoidal (see eq 3), while TEX_{86} is based only on the isoprenoidal ones (see eq 1). Given that the APCI conditions are usually not optimized simultaneously using both isoprenoidal and branched GDGTs, it is likely that values of BIT between various instruments are not comparable. Clearly much more investigation is needed to fully understand the ionization and transfer process of GDGTs in the APCI and the repercussions on the derived TEX_{86} and BIT indices, but we urge special caution when comparing BIT values between laboratories.

CONCLUSIONS

In this study, we show the applicability and investigate the reliability of alternatives to the common analytical protocols and equipment used to analyze archaeal and bacterial GDGTs in sediments for the measurement of the TEX_{86} and BIT indices. The cleanup experiments show that alkaline hydrolysis (saponification) is a valid alternative to the fractionation on activated alumina as long as the water extraction of the hexane fraction is avoided to minimize low recoveries of the GDGTs. Although alumina fractionation and alkaline hydrolysis are both widely used cleanup methods in paleoceanographic studies, alkaline hydrolysis might be especially useful to purify samples that are very rich in certain organic compounds. Thus, GDGT analyses can be readily performed in multiproxy biomarkers studies in samples which require saponification of the organic extracts. Regarding the low recovery of GDGT-0 when water is used to remove salts from hexane, we hypothesize that the GDGT might be preferentially solubilized by water due to the relative polarity of the compound. Silica and alumina used for column fractionation either activated or deactivated at 5% provide comparable results for TEX_{86} but not for BIT. The

degree of adsorbent activation seems to bias the BIT index and hence the use of nonactivated adsorbents is preferable. A reduction of flow in the HPLC after a change of HPLC columns with the same phase but different particle size, diameter, and manufacturer did not yield statistically significant differences of TEX_{86} and BIT values. Finally, TEX_{86} and BIT measurements on an ion-trap MS are compared for the first time to measurements obtained with a quadrupole MS. The ion-trap system is shown to be as precise as the quadrupole analyzer for both indices, and they also provide comparable TEX_{86} but significantly different BIT values. It appears that the ionization and transfer efficiency between branched and isoprenoidal GDGTs is different enough so that BIT values are very sensitive to MS operational conditions. This is especially important as at present much of the calibration work and provision of reference values to interpret the GDGT indices in a paleoclimatic context is derived from a single laboratory.^{7,17} Our results suggest that to use such reference values, the MS conditions should first be optimized so that the relative responses of the branched vs isoprenoidal GDGTs are comparable to the published reference values. This would be facilitated if a reference sample was available with which laboratories could tune their HPLC–MS systems to obtain comparable values among the increasing community analyzing the GDGT indices.

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