

Sensitivity Effects in $U^{k'}_{37}$ Paleotemperature Estimation by Chemical Ionization Mass Spectrometry

Roser Chaler, Joan O. Grimalt,* Carles Pelejero, and Eva Calvo

Department of Environmental Chemistry (ICER-CSIC), Jordi Girona, 18, 08034-Barcelona, Catalonia, Spain

Analysis of C_{37} alkenone mixtures by gas chromatography (GC) with flame ionization detection (FID) and GC coupled to mass spectrometry (GC/MS) in the chemical ionization mode (CI) shows that the later is useful for paleotemperature estimation when ammonia is used as reagent gas. Conversely, the use of isobutane gives rise to $U^{k'}_{37}$ readings that are dependent on the amount of C_{37} alkenones introduced in the system, being unreliable for paleoclimatic studies. However, ammonia CI GC/MS may produce $U^{k'}_{37}$ measurements that deviate from those obtained by GC-FID, the method calibrated for temperature estimation from algal cultures and marine sedimentary data. The differences result from changes in relative sensitivity between the di- and triunsaturated alkenones and depend on the instrument used and operational conditions. This problem is solved in the present study by determination of the response factor linear equations for each alkenone and their average relative sensitivity (R) using mixtures of known composition. These parameters allow the transformation of the GC/MS readings into the GC-FID equivalents using the following equation: $UG = R \times UM/(1 - UM(1 - R))$. Examples of the suitability of this approach are given.

The use of sedimentary C_{37} di- and triunsaturated ketones (heptatriaconta-15E,22E-dien-2-one and heptatriaconta-8E,15E,22E-trien-2-one, $C_{37:2}$ and $C_{37:3}$, respectively) for the estimation of sea surface temperatures (SST) during the Quaternary period¹ has been one of the most successful developments in organic geochemistry. This approach is based on the preferential synthesis of one or two unsaturated compounds by Haptophyceae in correspondence to the water temperature in which this alga grows.

A linear relationship between an unsaturation index, $U^{k'}_{37}$, expressed as

$$U^{k'}_{37} = 1/(1 + A) \quad (1)$$

where $A = C_{37:3}/C_{37:2}$ and growth temperature has been found from the study of the alkenone composition in cultures of

Emiliania huxleyi.² Linear $U^{k'}_{37}$ –SST relationships have also been found when comparing the sedimentary C_{37} alkenone composition and averaged SST records throughout the world oceans.^{3–5}

The high specificity of these alkenones as Haptophyceae biomarkers,⁶ their high concentrations in marine sediments,^{3,5,7} good resistance to biodegradation,⁸ and oxidative diagenesis,⁹ and the widespread abundance of the precursor organisms, namely, *E. huxleyi*,¹⁰ in the marine system¹¹ have promoted the increasing use of these alkenones as temperature estimators in paleoclimatic studies. Furthermore, temperature inferences from this estimator are simple since they depend on the relative composition of $C_{37:2}$ and $C_{37:3}$.

In principle, the analysis of these C_{37} alkenones in sedimentary samples does not require sophisticated instrumentation. They are usually major components of the solvent extracts, and their low volatility facilitates gas chromatographic separation from most sedimentary lipids. Therefore, they are currently analyzed by gas chromatography (GC) with flame ionization detection (FID) after some sample cleanup. The analytical requirements for the determination of the $U^{k'}_{37}$ index with this procedure have been considered in previous studies.^{12,13}

Alternatively, a method based on GC coupled to mass spectrometry (GC/MS) in chemical ionization mode (CI) has also been proposed.¹⁴ In this method, ammonia is used as reagent gas, taking advantage of the specific formation of ammonium adducts to alkenones. In contrast with the universal sensitivity properties of

- (2) Prah, F. G.; Wakeham, S. G. *Nature* **1987**, *330*, 367–369.
- (3) Rosell-Melé, A.; Eglinton, G.; Pflaumann, U.; Sarnthein, M. *Geochim. Cosmochim. Acta* **1995**, *59*, 3099–3107.
- (4) Pelejero, C.; Grimalt, J. O. *Geochim. Cosmochim. Acta* **1997**, *61*, 4789–4797.
- (5) Müller, P. J.; Kirst, G.; Ruhland, G.; von Storch, I.; Rosell-Melé, A. *Geochim. Cosmochim. Acta* **1998**, *62*, 1757–1772.
- (6) Marlowe, I. T.; Brassell, S. C.; Eglinton, G.; Green, J. C. *Chem. Geol.* **1990**, *88*, 349–375.
- (7) Villanueva, J.; Grimalt, J. O.; Cortijo, E.; Vidal, L.; Labeyrie, L. *Geochim. Cosmochim. Acta* **1997**, *61*, 4633–4646.
- (8) Sun, M.-Y.; Wakeham, S. G. *Geochim. Cosmochim. Acta* **1994**, *58*, 3395–3406.
- (9) Prah, F. G.; de Lange, G. J.; Lyle, M.; Sparrow, M. A. *Nature* **1989**, *341*, 434–437.
- (10) Conte, M. H.; Eglinton, G. *Deep-Sea Res. I* **1993**, *40*, 1935–1961.
- (11) Brassell, S. C. In *Organic Geochemistry*; Engel, M. H., Macko, S. A., Eds.; Plenum Press: New York, 1993; pp 699–738.
- (12) Villanueva, J.; Pelejero, C.; Grimalt, J. O. *J. Chromatogr., A* **1997**, *757*, 145–151.
- (13) Villanueva, J.; Grimalt, J. O. *Anal. Chem.* **1997**, *69*, 3329–3332.
- (14) Rosell-Melé, A.; Carter, J. F.; Parry, A. T.; Eglinton, G. *Anal. Chem.* **1995**, *67*, 1283–1289.

* Corresponding author: (e-mail) jgoqam@cid.csic.es.

(1) Brassell, S. C.; Eglinton, G.; Marlowe, I. T.; Pflaumann, U.; Sarnthein, M. *Nature* **1986**, *320*, 129–133.

FID, quantification in this case is based on an alkenone-dependent specific signal, potentially involving higher selectivity. This method is particularly suited for paleotemperature studies in areas with low sedimentary alkenone content where these compounds are minor constituents of the solvent-extractable neutral lipid fraction such as the cold regions at high latitudes. In these cases, the C_{37} alkenone peaks do not stand out in the GC-FID trace and paleotemperature estimation is strongly complicated, requiring intensive cleanup procedures¹² for the elimination of coeluting compounds.

Unfortunately, the change from universal to specific detectors may involve some problems of paleotemperature determination when sensitivity differences between $C_{37:2}$ and $C_{37:3}$ are encountered. As shown in the present study, these sensitivity differences depend on the instrumental set used for the analyses. Results obtained with three different CI GC/MS instruments are reported for description of this problem. In addition, two of these instruments were operated with isobutane for comparison.

Nevertheless, the ammonia CI GC/MS approach can be used for paleotemperature estimation in these nonlinear conditions provided that the sensitivity ratio among both compounds is determined and adequate transformation equations are used. The equations required for this transformation are also developed in the present study.

THEORY

One basic requirement of analytical instrumentation is the linearity between signal area (S) and analyte concentration (C):

$$S = aC + b \quad (2)$$

where a and b are constants for a concentration range.

The measurement of $U^{k'}_{37}$ involves two compounds, $C_{37:2}$ and $C_{37:3}$, which can be measured by GC or GC/MS. Thus, four sensitivity constants, a_{2G} , a_{3G} , a_{2M} , and a_{3M} , have to be considered

$$S_{2G} = a_{2G}C_{37:2} + b_{2G} \quad (3)$$

$$S_{3G} = a_{3G}C_{37:3} + b_{3G} \quad (4)$$

$$S_{2M} = a_{2M}C_{37:2} + b_{2M} \quad (5)$$

$$S_{3M} = a_{3M}C_{37:3} + b_{3M} \quad (6)$$

$U^{k'}_{37}$ is theoretically defined as in eq 1. However, the instrumental determination of this index is currently performed from direct measurement of signal areas recorded by GC (most cases) or GC/MS. Thus, the important question is how to get true concentration ratios from the measured peak ratios. In practical terms, equations relating the $U^{k'}_{37}$ indexes calculated from these two types of signal areas, UG and UM, respectively, need to be developed. From eqs 3–6

$$UG = S'_{2G}/(S'_{2G} + S'_{3G}) = a_{2G}C_{37:2}/(a_{2G}C_{37:2} + a_{3G}C_{37:3}) \quad (7)$$

$$UM = S'_{2M}/(S'_{2M} + S'_{3M}) = a_{2M}C_{37:2}/(a_{2M}C_{37:2} + a_{3M}C_{37:3}) \quad (8)$$

where $S'_i = S_i - b_i$, for $i = 2G, 3G, 2M$, and $3M$.

From eqs 7 and 8

$$C_{37:3}/C_{37:2} = (a_{2G}/a_{3G})(1/UG - 1) = (a_{2M}/a_{3M})(1/UM - 1) \quad (9)$$

Equation 9 relates the $U^{k'}_{37}$ indexes calculated from GC and GC/MS signal areas for the generalized case in which $C_{37:3}$ and $C_{37:2}$ have different sensitivity constants. This equation can be converted into more operational expressions such as

$$UM = UG/(R + UG(1 - R)) \quad (10)$$

$$UG = R \times UM/(1 - UM(1 - R)) \quad (11)$$

where $R = (a_{2G}a_{3M})/(a_{2M}a_{3G})$.

The R value can be defined as relative sensitivity. This term is constant as consequence of the proportionality rule between signal and compound concentration (eqs 2–6). Currently, a_{2G}/a_{3G} will be equal to unity because of the universal properties of the flame ionization detector. Thus, R will normally depend on the a_{2M}/a_{3M} values, that is, the GC/MS sensitivity ratio between di- and triunsaturated alkenones. Thus, in practical terms

$$R = a_{3M}/a_{2M} \quad (12)$$

although this restriction is not needed for the present mathematical development.

As indicated by eqs 10 and 11, the correspondence between UG and UM is not linear (Figure 1). The deviations between both values are higher at medium $U^{k'}_{37}$ values ($U^{k'}_{37} \sim 0.3\text{--}0.5$) and minimal at the extremes ($0.1 > U^{k'}_{37} > 0.9$). The differences are higher as highest is the difference in sensitivity between both alkenones. Obviously, for $R = 1$, both $U^{k'}_{37}$ are identical.

Once the instrumental conditions have been established, e.g., calibration, reagent gas pressure, etc., the R constant can be calculated from the measurement of UG and UM from a series of alkenone standards or reference samples. For this purpose, eq 10 can be transformed into the following form:

$$R = (UG - UM \times UG)/(UM - UM \times UG) \quad (13)$$

Thus, calculation of R for each alkenone mixture of the series of standards provides different values that can be finally averaged. The uniformity of these values provide an estimation of the linearity of the $C_{37:3}$ and $C_{37:2}$ alkenone signals under the instrumental conditions selected for analysis.

Since all equations relating $U^{k'}_{37}$ and SST have been calibrated with GC measurements,^{2–5} deviations of UM from UG will involve estimation errors that need to be evaluated. These can be calculated from eq 10 or 11.

$$E = UM - UG \quad (14)$$

and

$$E = UM(1 - R)(1 - UM)/(1 - UM(1 - R)) \quad (15)$$

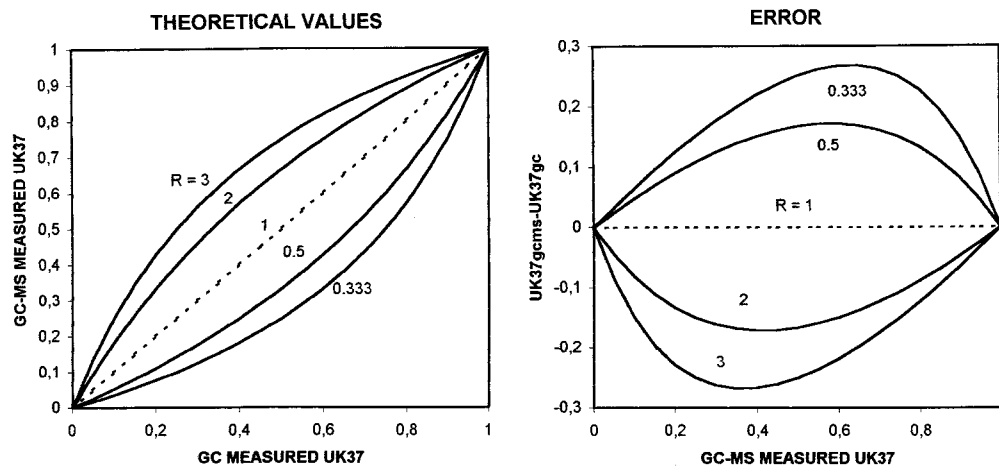


Figure 1. Theoretical differences in U_{37}^k measurements by GC-FID and GC/MS (eq 10) for $C_{37:2}$ and $C_{37:3}$ alkenones exhibiting different relative sensitivities to GC/MS (R between 0.333 and 3; eq 12). The error function is calculated as described in eq 15.

E provides the differences between both measurements in U_{37}^k relative units, UM in this case. These differences depend on the U_{37}^k readings (Figure 1) since different proportions of both alkenones are involved in each measurement. As expected, E is larger as R is deviates more from unity. Thus, GC/MS instruments will provide larger deviations from the GC measurements the higher their sensitivity difference for $C_{37:2}$ and $C_{37:3}$.

EXPERIMENTAL SECTION

Materials. Residue-analysis isooctane was from Merck (Darmstadt, FRG). The synthetic alkenone standards, heptatriaconta-15 E ,22 E -dien-2-one and heptatriaconta-8 E ,15 E ,22 E -trien-2-one, were kindly provided by Prof. Maxwell (Organic Geochemistry Group, University of Bristol).¹⁵

Sample Handling. After addition of an internal standard of n -hexatriacontane, sediments (2–3 g) were extracted three times in an ultrasonic bath (20 min) with 3×10 mL of dichloromethane. The extracts were collected together and evaporated to dryness under a nitrogen stream. The evaporated extracts were dissolved with 3 mL of 6% potassium hydroxide in methanol and stored overnight. Nonacidic compounds were recovered by extraction with n -hexane (3×3 mL). The resulting extracts were cleaned by stirring with water (1 mL) for the elimination of possible KOH traces. Prior to instrumental analysis, the extracts were concentrated under a gentle stream of nitrogen and derivatized overnight with bis(trimethylsilyl)trifluoroacetamide at room temperature.

Gas Chromatography. GC analyses were performed using a Varian STAR 3400 CX (Varian, CA) equipped with a septum programmable injector and a flame ionization detector. Alkenones were separated in a 50 m $\times$ 0.32 mm i.d., 0.12- μ m film thickness CPSIL-5 CB column (Chrompack, Middleburg, The Netherlands). Hydrogen was the carrier gas (50 cm/s). Oven temperature was programmed from 90 (held 1 min) to 140 $^{\circ}$ C at 20 $^{\circ}$ C/min, then to 280 $^{\circ}$ C at 6 $^{\circ}$ C/min (held 25 min), and finally to 320 $^{\circ}$ C at 10 $^{\circ}$ C/min (held 6 min). The injector was programmed from 90 (held 0.3 min) to 320 $^{\circ}$ C at 200 $^{\circ}$ C/min (final holding time 55 min). Detector temperature was 320 $^{\circ}$ C.

Gas Chromatography/Mass Spectrometry. Three GC/MS instruments were used for the analyses of the C_{37} alkenones in

the chemical ionization mode: (1) Varian STAR 3400 coupled with a Finnigan INCOS XL, (2) Fisons MD 800 (ThermoQuest, Manchester, U.K.), and (3) Hewlett-Packard HP5890 II coupled to a HP5988A (Palo Alto, CA). The gas chromatographs of these instruments were equipped with a 30 m $\times$ 0.25 mm i.d. HP-5-MS column (0.25- μ m film thickness) and a split/splitless injector. Injection (280 $^{\circ}$ C) was in splitless mode (hot needle technique) keeping the split valve closed for 48 s (1.5 min in instrument 3). Helium was used as carrier gas (1 mL/min). In instrument 1, oven temperature was programmed from 90 (held 1 min) to 300 $^{\circ}$ C at 15 $^{\circ}$ C/min (held 25 min). In instruments 2 and 3, oven temperatures were programmed from 90 (held 1 min) to 120 $^{\circ}$ C at 15 $^{\circ}$ C/min and then to 310 $^{\circ}$ C at 10 $^{\circ}$ C/min (held 20 min).

Transfer line temperatures were 300 (instruments 1 and 3) and 280 $^{\circ}$ C (instrument 2). Ion source temperatures were 130 (instrument 1) and 150 $^{\circ}$ C (instruments 2 and 3). Mass fragmentograms were recorded in positive chemical ionization mode using ammonia ($m/z = 546, 548$; instruments 1–3) and isobutane ($m/z = 511, 513, 529, 531$; instruments 1 and 3). Instruments 1 and 3 were also operated in full scan. Ion source reagent gas pressures were adjusted to obtain the maximum yield of the pseudomolecular ions of the C_{37} alkenones, 1.8 and 1 Torr for ammonia and isobutane, respectively.

RESULTS AND DISCUSSION

Mass Spectral Data. The CI mass spectra obtained with ammonia and isobutane as reagent gases are reported in Figure 2. The former are constituted by the pseudomolecular ion $[M + NH_4]^+$ (m/z 548 and 546 for $C_{37:2}$ and $C_{37:3}$, respectively) without any other significant fragmentation. These spectra are coincident with those previously reported,¹⁴ particularly for $C_{37:2}$. In this previous study, the mass spectra of $C_{37:3}$ exhibited a $[M - 18]^+$ fragment of low intensity ($\sim 8\%$) which is not observed in Figure 2.

The isobutane mass spectra are also dominated by the pseudomolecular ions $[M + H]^+$ (m/z 531 and 529 for $C_{37:2}$ and $C_{37:3}$, respectively), but in contrast, both compounds exhibit fragments of significant intensity such as $[M - H_2O + H]^+$ (m/z 513 and 511 for $C_{37:2}$ and $C_{37:3}$, respectively). The relative intensity of this fragment is considerably higher in the case of $C_{37:3}$.

(15) Rechka, J. A.; Maxwell, J. R. *Org. Geochem.* **1988**, *13*, 727–734.

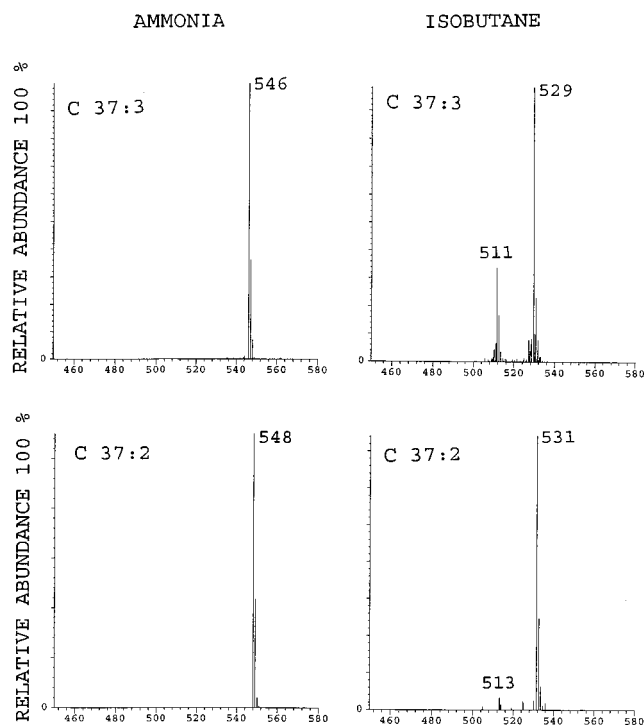


Figure 2. Mass spectra of the $C_{37:2}$ and $C_{37:3}$ alkenones under chemical ionization with ammonia and isobutane as reagent gases.

Table 1. Relative Sensitivities of the C_{37} Alkenones ($C_{37:3}/C_{37:2}$) upon Analysis with GC/MS CI Mode Using Ammonia and Isobutane as Reagent Gases

instrument	reagent gas			
	ammonia		isobutane	
	10 ng ^a	100 ng ^a	10 ng ^a	100 ng ^a
MD800	0.56	0.54		
HP5988	0.76	0.76	0.43	0.21
INCOS-XL	0.96	1.0	0.62	0.31

^a Total amount of C_{37} .

The use of these specific pseudomolecular ions in mass fragmentography provides higher selectivity than GC-FID. They allow the quantification of the alkenones even when they are hardly detectable in the GC profiles.

Sensitivity Effects. Significant changes in relative sensitivity between both C_{37} alkenones are observed when analyzed by the above-described CI GC/MS methods (Table 1). The variability is higher with isobutane, which is probably a consequence of the higher fragmentation of $C_{37:3}$ under ionization with this reagent gas (Figure 2). Furthermore, comparison of the results obtained for different alkenone amounts introduced in the system (Table 1) shows a dependence of relative sensitivities from total injected alkenones. In contrast, ammonia provides lower sensitivity deviations, probably as a consequence of the lower alkenone fragmentation with this reagent gas (Figure 2). In this case, no significant dependence between relative sensitivity and alkenones injected into the system is observed.

According to the Theory section, these changes in relative sensitivity are reflected in U'_{37} deviations, which increase with the magnitude of the response factor differences of the two

alkenones. Therefore, CI GC/MS measurement of the C_{37} alkenone composition will lead to values deviating from those obtained from the analysis of the same mixtures with GC FID (the most currently used method). These sensitivity effects are illustrated in Figure 3 for a standard alkenone mixture ($U'_{37} = 0.736$), which was repeatedly analyzed at several concentration ranges.

As evidenced in this figure, the use of isobutane as reagent gas gives rise to a strong dependence of the resulting U'_{37} values from alkenone amount. This dependence greatly complicates the use of CI GC/MS with isobutane for paleotemperature estimation with the alkenone method since a_{2M} and a_{3M} in eqs 5 and 6 are not constant in this case.

Ammonia also provides values deviating from the GC FID determinations (Figure 3), but in contrast, they do not exhibit significant dependence from the alkenone amount introduced in the system. These constant values can be used for paleotemperature estimation provided that adequate corrections for instrumental effects are introduced, e.g., ion source temperature and reagent gas pressure.

In terms of U'_{37} dispersion, the CI GC/MS exhibits lower or equal standard deviation than GC measurements when isobutane and ammonia are used (Figure 3). This aspect also facilitates the use of CI GC/MS with ammonia for paleotemperature estimation.

Correction of Ammonia CI GC/MS and GC-FID Sensitivity Differences. Further assessment on the usefulness of the previously reported equations is provided by ammonia CI GC/MS and GC-FID analysis of a series of standard mixtures covering most of the U'_{37} range. However, this comparison requires not only the measurement of the relative sensitivity coefficient (R ; eqs 12 and 13) but the intercept of the response factor linear equations (b; eqs 3–6). As shown in Figure 4, the linear relation between analyte amount and CI GC/MS area signal has a significant intercept that normally exhibits negative values (in the order of 35–55% of the slope signal at 5 ng). This term is also observed in GC-FID (eqs 3 and 4) although at significantly lower values (on the order of 5–15% of the slope signal at 5 ng). In this later case, this term is consistent with the gas chromatographic adsorption effects described in a previous study.¹³ In contrast, the higher intercept value of CI GC/MS indicates the concurrence of other effects reinforcing its significance in the response factor equations (eqs 5 and 6).

In practice, once the instrumental conditions are defined, U'_{37} measurement by CI GC/MS requires the calibration of the response factor with $C_{37:2}$ and $C_{37:3}$ standards (Figure 4) for inclusion of the intercept in the calculations (eqs 7 and 8). This calibration is also recommended for GC-FID analysis although in this latter case this requirement is not so important due to the lower relative proportion of the intercept. However, the analyst should be aware of the operational conditions avoiding deviations of the U'_{37} measurements related to adsorption effects.¹³

The good agreement between theoretical and experimental results is shown in Figure 5 for a series of standard mixtures covering a wide range of U'_{37} indexes. These mixtures have been analyzed with various CI GC/MS instrumental settings (Figure 3) giving rise to relative sensitivity indexes (R) such as 0.72, 1.05, and 1.42 upon comparison of the UM and UG values. These R values are currently encountered in the analysis of alkenones by

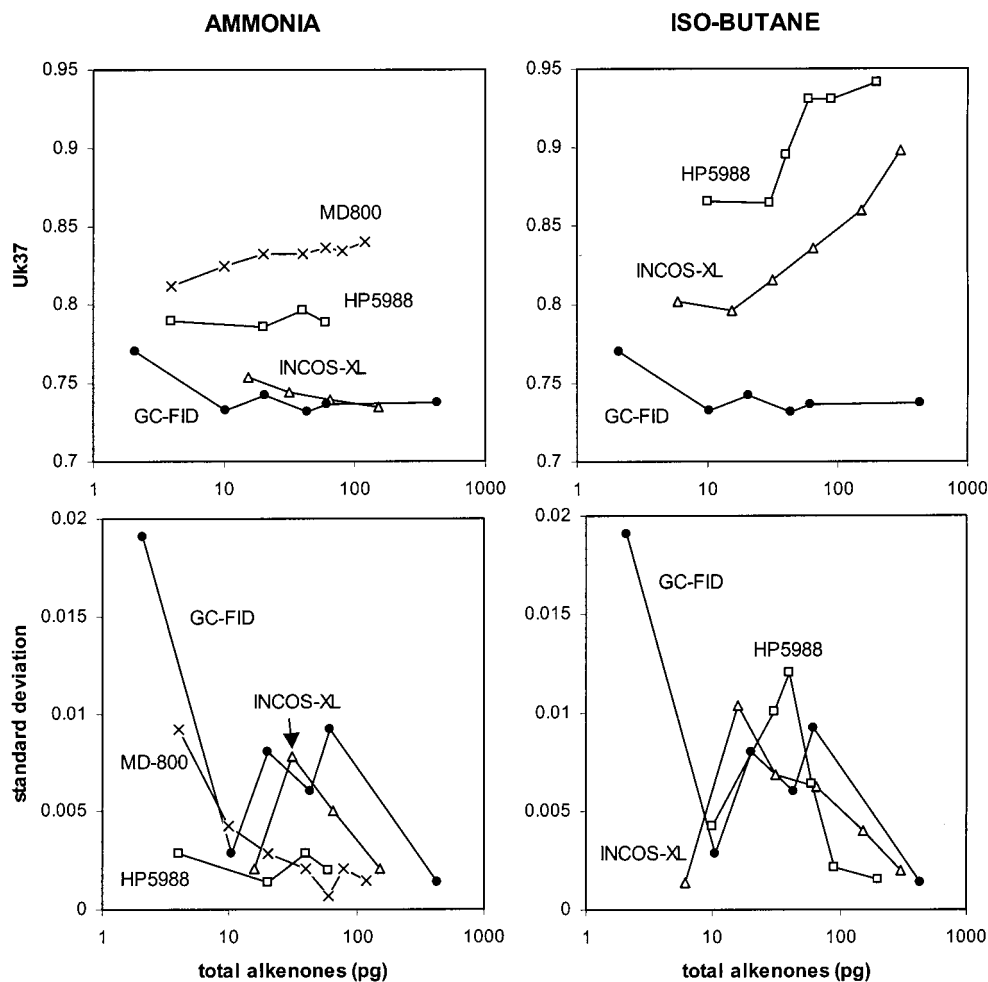


Figure 3. U_{K37} indexes obtained from the analysis of a standard alkenone mixture ($U_{K37} = 0.736$) using several GC/MS instruments with ammonia and isobutane as reagent gases. The results obtained with GC-FID are also shown for comparison.

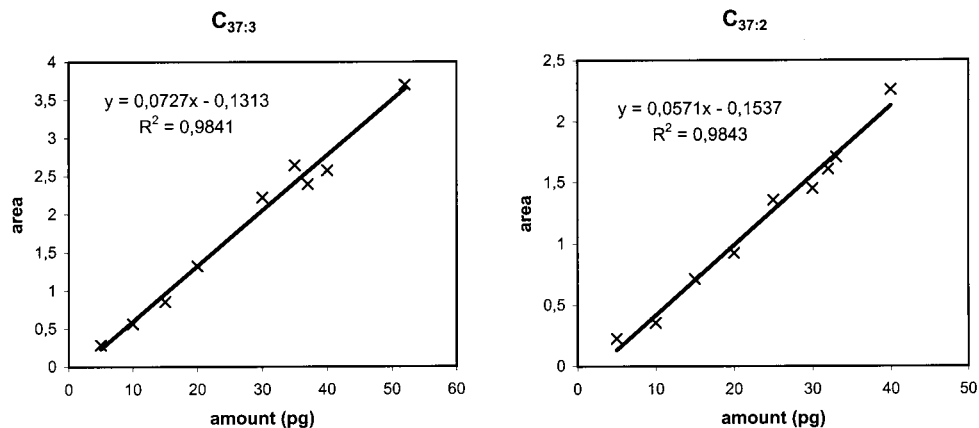


Figure 4. Curve-fitted straight lines showing the linearity of the response factor upon analysis of $C_{37:3}$ and $C_{37:2}$ by ammonia CI GC/MS. The independent ordinate is significant at low alkenone amounts.

CI GC/MS. The equations developed in this study are also useful for the prediction of the differences between UM and UG indexes (Figure 5). As indicated by eq 15, the differences are higher at intermediate U_{K37} values where deviations up to 0.09 can be observed. These deviations are important since they represent intervals of 2.7 °C when transformed into paleotemperatures,^{2,5} and this difference is very relevant in terms of paleoclimatic interpretation.

In any case, the nonlinear relationship between the two instrumental U_{K37} indexes can be described by the equations developed in the present study and does not involve any restriction for the use of ammonia CI GC/MS for paleotemperature determination. Some examples of their application to samples from the North Atlantic Ocean, the Equatorial Pacific Ocean, and the South China Sea are shown in Table 2. In all cases, transformation of UM into UG using eq 11 and comparison with UG obtained by

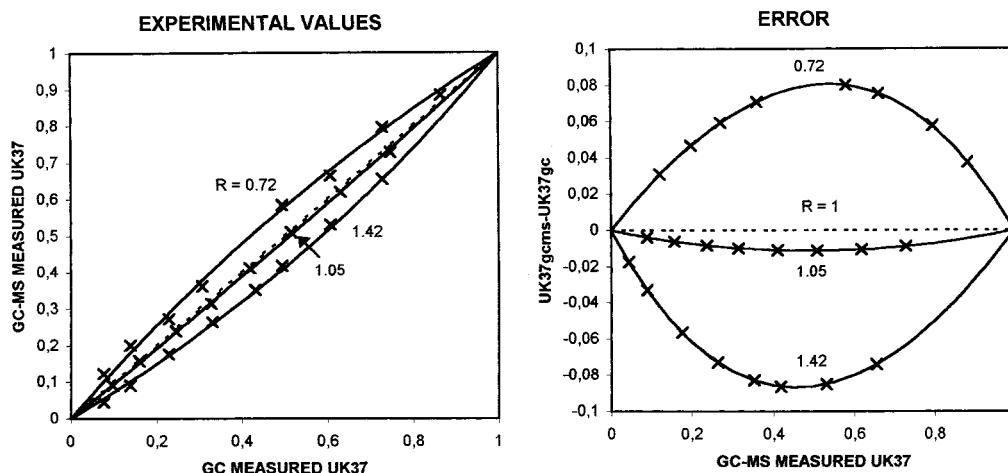


Figure 5. Comparison of the differences in $U^{k'}_{37}$ values resulting from the analysis of alkenone mixtures with GC-FID and ammonia CI GC/MS (x points). The results are plotted together with the theoretical curves (solid line) obtained from eqs 11 and 15 after calculation of the average relative sensitivity (eq 13).

Table 2. Correction of the $U^{k'}_{37}$ Values Measured by Ammonia CI GC/MS to the Equivalent GC Determinations

sample	site	GC/MS values	transformation to GC ^a	GC readings	difference
MD952037 1520 cm	North Atlantic Ocean	0.5730	0.5518	0.5533	0.0014
MD952037 1348 cm	North Atlantic Ocean	0.6510	0.6312	0.6236	0.0076
XX365	Equatorial Pacific Ocean	0.6759	0.6568	0.6528	0.0040
17964–2 260 cm	South China Sea	0.8955	0.8872	0.8874	0.0003

^a Using eq 11 in the Theory section.

direct GC-FID measurement gives rise to deviations well below 0.0165, the alkenone range for 0.5 °C error.¹³

CONCLUSIONS

CI GC/MS with ammonia as reagent gas is an adequate technique for paleotemperature estimation in samples requiring a selective analysis of C_{37} alkenones due to interfering compounds. The use of this technique may give rise to $U^{k'}_{37}$ measurements that deviate from those obtained by GC-FID. The differences are due to the changes in CI GC/MS relative sensitivity between the two alkenone compounds, $C_{37:2}$ and $C_{37:3}$, and depend from the instrument used and operational conditions. However, these GC/MS values can easily be transformed into the GC-FID equivalents using the equations developed in the present study. For this purpose, the instrument should be calibrated with a reference series of standards encompassing the same or a larger $U^{k'}_{37}$ range as that in the samples of study. This series must be used to determine the constants of the response factor linear equations, namely, the intercept and the average relative sensitivity (eq 13), which are the constants involved in the transformation equation (eq 11).

Conversely, CI GC/MS with isobutane as reagent gas does not provide independent $U^{k'}_{37}$ measurements from the amount of C_{37} alkenones introduced in the system. The higher fragmentation of $C_{37:3}$ with respect to $C_{37:2}$ involves strong changes in relative sensitivity, and the resulting $U^{k'}_{37}$ measurements are not useful for paleotemperature estimation.

ACKNOWLEDGMENT

Financial support from the TEMPUS (ENV4-CT97-0564) and the HOLOCENE (ENV4-CT97-0643) EU projects is acknowledged. This work was also financed by the Spanish Council for scientific research (CICYT; CLI98-1002-CO2-01). C.P. thanks CIRIT (Generalitat de Catalunya) for a Ph.D. grant.

Received for review August 23, 2000. Accepted September 11, 2000.

AC001014Q