

Non-linear effects in the determination of paleotemperature $U_{37}^{k'}$ alkenone ratios by chemical ionization mass spectrometry[☆]

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Abstract

The performance of gas chromatography coupled to mass spectrometry in the positive chemical ionization mode using ammonia as reagent gas (GC–PCI–MS) in the analysis of C_{37} alkenones for paleotemperature estimation has been re-evaluated. In some conditions, the discrepancies observed in the measurement of the $U_{37}^{k'}$ index with this technique as compared with GC equipped with flame ionization detection (GC–FID) cannot be explained by differences in sensitivity between the tri- and diunsaturated alkenones. Thus, at low (currently <0.3) or high (currently >0.4) $U_{37}^{k'}$ values the GC–PCI–MS determinations may be observed to be lower or higher, respectively, than those measured with GC–FID. As shown by analysis of a series of synthetic C_{37} alkenone standards these discrepant results can be explained by non linear effects in the GC–PCI–MS response factors. Second-order polynomial functions provide equations that describe better the signal to amount of analyte ratios. Users of GC–PCI–MS should calibrate their instruments with standards of known C_{37} alkenone composition in order to minimize non-linear effects.

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1. Introduction

The use of sedimentary C_{37} di- and triunsaturated ketones, heptatriaconta-15*E*,22*E*-dien-2-one ($C_{37:2}$) and heptatriaconta-8*E*,15*E*,22*E*-trien-2-one ($C_{37:3}$), for the estimation of sea surface temperatures (SSTs) during the Quaternary has been one of the most

successful achievements in organic geochemistry [1]. This approach is based on the preferential synthesis of these unsaturated compounds by haptophyte algae in correspondence to the water temperature where they grow. Thus, when the water temperature decreases, algae synthesize mixtures with a higher proportion of the triunsaturated alkenone.

An unsaturation index, $U_{37}^{k'}$, is defined from the relative composition of these alkenones, $C_{37:2}$ and $C_{37:3}$:

$$U_{37}^{k'} = (C_{37:2}) / [(C_{37:2}) + (C_{37:3})] \quad (1)$$

An empirical relationship between this unsatura-

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tion index, $U_{37}^{k'}$, and temperature has been established based on studies of the alkenone composition in cultures of algae *Emiliana huxleyi* grown at different temperatures [2]:

$$T = (U_{37}^{k'} - 0.043)/0.033 \quad (2)$$

This linear relationship has also been observed when comparing the sedimentary C_{37} alkenone composition and averaged overlying SST records throughout the world oceans [3–5].

In addition, these alkenones have other interesting properties that facilitate their widespread use in paleoclimatic studies, they are specific biomarkers of haptophyceae algae [6], being found in most marine systems due to the widespread occurrence of these precursor organisms [7]. These compounds also exhibit high resistance to biodegradation [8] and this process currently does not involve $U_{37}^{k'}$ changes [9]. The combined effect of widespread haptophyceae occurrence and resistance to biodegradation generally involves the dominance of these alkenones in the lipid extracts of marine sediments [10].

Accordingly, these alkenones are currently analyzed by gas chromatography with flame ionization detection (GC–FID) [11,12]. In addition, GC coupled to mass spectrometry (GC–MS) in the positive chemical ionization (PCI) mode using ammonia as reagent gas has been proposed as an alternative technique for sediments with low alkenone concentrations or with a lot of interfering compounds [13]. This technique is based on the specific binding of ammonia to keto groups [13] since selective ion recording (SIM) of the pseudomolecular ions $[M + NH_4]$ (m/z 548 and m/z 546 for $C_{37:2}$ and $C_{37:3}$, respectively) allows the quantification of the alkenones present in the extracts and the subsequent determination of the $U_{37}^{k'}$ index. The usefulness of this approach has been reported in previous studies [13,14] but the influence of sensitivity effects due to the different response factor of each alkenone has to be considered [14].

However, the use of this GC–MS technique is based on the assumption of linear response factor equations. Otherwise the measurement of the $U_{37}^{k'}$ index may be compromised. In a previous study, the discrepancies in the measurement of $U_{37}^{k'}$ with GC–MS and GC–FID due to sensitivity effects in the MS

systems were considered assuming a linear model, now the difficulties associated with deviations from linearity of the GC–MS instrument are described.

2. Experimental section

2.1. Chemicals

Residue-analysis-grade isooctane (Ref. 1.15440) was from Merck (Darmstadt, Germany). Synthetic alkenone standards, $C_{37:2}$ and $C_{37:3}$, were kindly provided by Professor Maxwell (Environmental and Analytical Chemistry Section, University of Bristol, Bristol, UK) [15].

2.2. Instrumental analysis

GC analyses were performed using a Varian STAR 3400 CX (Varian, CA, USA) equipped with a septum programmable injector and an FID system. Alkenones were separated in a 50 m×0.32 mm I.D., 0.12 μ m film thickness, CPSIL-5 CB column (Chrompack, Middelburg, The Netherlands). Hydrogen was the carrier gas (50 cm/s). Oven temperature was programmed from 90 (held 1 min) to 140 °C at 20 °C/min, then to 280 °C at 6 °C/min (held 25 min), and finally to 320 °C at 10 °C/min (held 6 min). The injector was programmed from 90 °C (held 0.3 min) to 320 °C at 200 °C/min (final holding time 55 min). Detector temperature was 320 °C.

An Agilent 6890A coupled to a HP5973 (Agilent, Palo Alto, CA, USA) (A) and a Fisons MD800 (Thermo Quest, Manchester, UK) (B) were used for the GC–MS analyses. Both instruments were equipped with a 30 m×0.25 mm I.D. HP5-MS non-polar fused-silica capillary column (film thickness: 0.25 μ m). Helium was used as carrier gas (1.0 ml/min). Oven temperature was programmed from 90 °C (held 1 min) to 120 °C at 12 °C/min and then to 320 °C at 10 °C/min, with an isothermal hold for 10 min. Injection was in the splitless mode (hot-needle technique). The split valves were closed for 48 and 90 s in the Fisons and Agilent instruments, respectively. The injector temperature was 280 °C in both cases. Ion source and transfer-line temperatures

were 200 °C (230 °C in the Agilent instrument) and 280 °C, respectively. Mass fragmentograms were recorded in the PCI mode (m/z 544, 545, 546, 547, 548, 549, 550, 551, 552) using ammonia as reagent gas (gas pressure $1.3 \cdot 10^{-4}$ Torr; 1 Torr = 133.3222 Pa). Mass-Lab (Thermo Quest) and Enhanced Chemstations G1701CA software were used for data acquisition and analysis in the Fisons and Agilent instruments, respectively.

3. Results and discussion

3.1. Response factor equations

The analysis of $C_{37:3}$ and $C_{37:2}$ by GC–FID usually provides a linear response (Fig. 1). The straight lines exhibit very similar slopes for both alkenones and a higher negative intercept for $C_{37:3}$ than $C_{37:2}$ [16], which probably reflects the higher adsorption of the triunsaturated alkenone in the GC columns [12]. The similar slopes are consistent with the universal sensitivity and general nonselectivity of FID as well as the close chemical composition of both alkenones. As described previously [16], the differences in intercept values may involve deviations between recorded and true $U_{37}^{k'}$ values at low sample amounts. In the present study, sufficient alkenone concentrations have been analysed in all cases in order to avoid this distortion effect.

The analysis of these alkenones by GC–PCI-MS

currently involves different slopes and therefore deviations from the $U_{37}^{k'}$ indices obtained by GC–FID [14]. As described recently [14], reliable $U_{37}^{k'}$ measurements may be obtained despite these differences provided that the ratio between signal (S) and analyte amount (C) is linear. However, in some cases, S – C relationships other than linear are observed. As shown in Fig. 2, representation of signal area vs. sample amount may exhibit significant deviations from linearity, giving rise to good curve fits to a second-order polynomial expression.

In these conditions, the signal areas of $C_{37:3}$ and $C_{37:2}$ upon analysis with the GC–MS system, S_{3M} and S_{2M} , respectively, can be expressed as:

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

$$S_{2M} = c_{2M}C_{37:2}^2 + b_{2M}C_{37:2} + a_{2M} \quad (4)$$

where a_{3M} , b_{3M} and c_{3M} and a_{2M} , b_{2M} and c_{2M} are the polynomial coefficients for $C_{37:3}$ and $C_{37:2}$, respectively.

The signal areas of $C_{37:3}$ and $C_{37:2}$ for GC–FID, S_{3G} and S_{2G} , respectively, are as follows:

$$S_{3G} = b_{3G}C_{37:3} + a_{3G} \quad (5)$$

$$S_{2G} = b_{2G}C_{37:2} + a_{2G} \quad (6)$$

where b_{3G} and a_{3G} and b_{2G} and a_{2G} are the linear coefficients for $C_{37:3}$ and $C_{37:2}$, respectively.

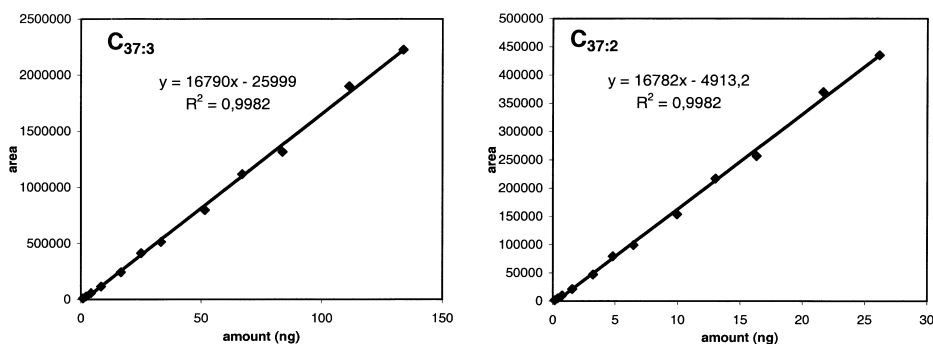


Fig. 1. Representative examples of the response factor equations obtained in the gas chromatographic analysis of the $C_{37:3}$ and $C_{37:2}$ alkenones using a flame ionization detector.

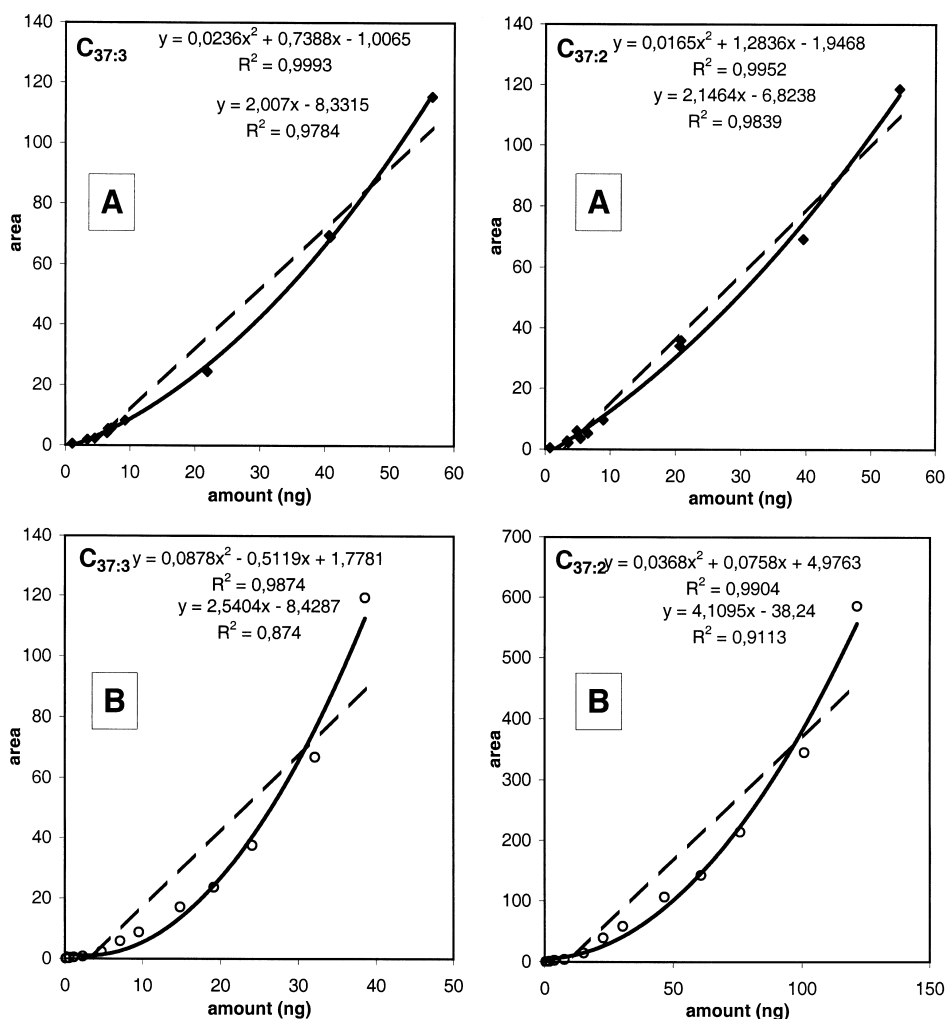


Fig. 2. Representative examples of the response factor equations obtained in the gas chromatographic analysis of the $C_{37:3}$ and $C_{37:2}$ alkenones using the positive ion chemical ionization mass spectrometry set ups considered in this study. A and B refer to different instruments. Broken and continuous lines correspond to curve fitting with straight lines and second-order polynomials, respectively.

3.2. GC-FID $U_{37}^{k'}$ and GC-MS $U_{37}^{k'}$ indices

According to the previous discussion Eqs. (5) and (6) can be simplified assuming that $b_{3G} = b_{2G}$ due to the nonselectivity of FID. a_{3G} and a_{2G} can also be omitted because, for simplicity, sufficient alkenone concentrations have been taken in order to avoid adsorption effects [16]. In these conditions, Eq. (1) reflects the $U_{37}^{k'}$ expression resulting from GC-FID.

Eqs. (3) and (4) can be used to obtain a mathe-

tical expression of $U_{37}^{k'}$ as calculated from GC-MS measurements:

$$U_{37}^{k'} = (c_{2M}C_{37:2}^2 + b_{2M}C_{37:2} + a_{2M}) / (c_{2M}C_{37:2}^2 + b_{2M}C_{37:2} + a_{2M} + c_{3M}C_{37:3}^2 + b_{3M}C_{37:3} + a_{3M}) \quad (7)$$

Thus, using Eq. (7) and the curve fitted constants from Fig. 2, GC-MS $U_{37}^{k'}$ can be represented vs.

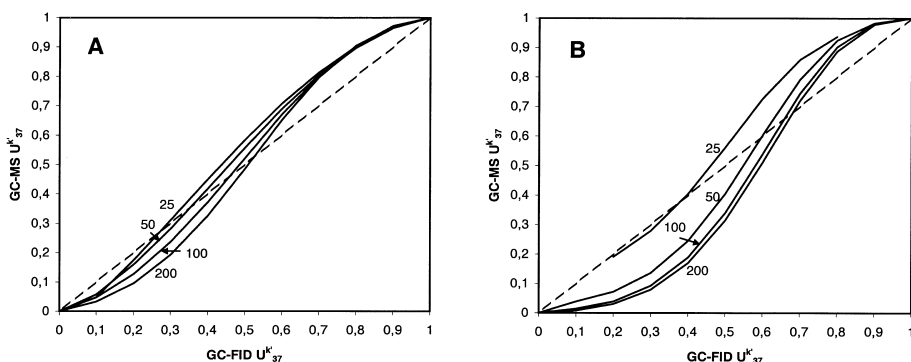


Fig. 3. Predicted differences between $U_{37}^{k'}$ indices recorded by gas chromatography coupled to flame ionization detection and mass spectrometry in the positive ion chemical ionization mode as calculated from Eqs. (1) and (7). Numbers refer to total alkenones in ng. A and B refer to the curve fitted lines reported for the two instruments involved in Fig. 2. The broken line corresponds to $GC-FID U_{37}^{k'} = GC-MS U_{37}^{k'}$ and is given for reference.

$GC-FID U_{37}^{k'}$. As shown in Fig. 3, the relationship between both indices depends on alkenone amounts but at high values the curves exhibit similar trends.

The most distinct feature concerns the inflexion point (IP) at which $GC-MS U_{37}^{k'}$ have higher values than $GC-FID U_{37}^{k'}$ and vice versa which may occur at values higher or lower than $U_{37}^{k'} = 0.5$. In any instance, the occurrence of this IP involves a major difference in relation to the curves obtained from comparison of $GC-MS U_{37}^{k'}$ and $GC-FID U_{37}^{k'}$ under linear conditions when the response of the $GC-MS$ system is linear no IP is observed and therefore $GC-MS U_{37}^{k'}$ is either higher or lower than $GC-FID U_{37}^{k'}$ over the entire 0–1 range [14].

The plots calculated in Fig. 3 are similar to real case comparisons such as the one in Fig. 4 where the $GC-MS U_{37}^{k'}$ and $GC-FID U_{37}^{k'}$ data measured over a series of standard mixtures are displayed. Again, an inflexion point is observed. At $GC-FID U_{37}^{k'}$ values > 0.4 these are lower than the $GC-MS U_{37}^{k'}$ values. In contrast, at $GC-FID U_{37}^{k'} < 0.4$ these are higher than the $GC-MS U_{37}^{k'}$ values.

3.3. Inflexion point

The above described equations can be used to locate the position of the inflexion point (IP). The terms a_{3M} and a_{2M} in Eqs. (3) and (4), respectively, also represent contributions from background or

adsorption effects. Thus, they can also be assimilated to 0 at sufficient high analyte concentrations. Thus, Eqs. (3) and (4) can be re-written as:

$$S_{3M} = c_{3M} C_{37:3}^2 + b_{3M} C_{37:3} \quad (8)$$

$$S_{2M} = c_{2M} C_{37:2}^2 + b_{2M} C_{37:2} \quad (9)$$

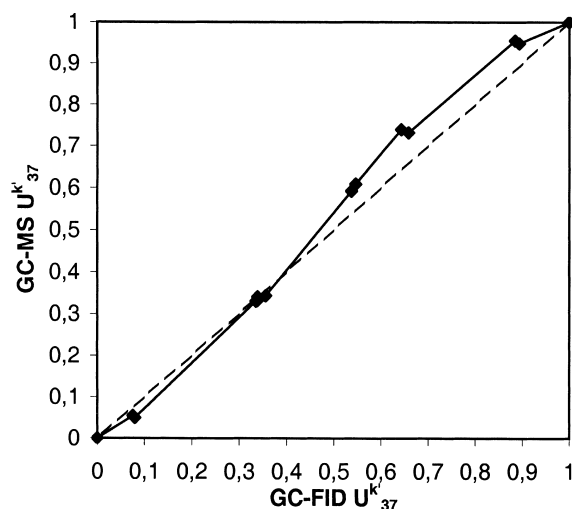


Fig. 4. Differences between $U_{37}^{k'}$ indices measured by gas chromatography coupled to flame ionization detection and mass spectrometry in the positive ion chemical ionization mode measured over a series of C_{37} standard mixtures. The broken line corresponds to $GC-FID U_{37}^{k'} = GC-MS U_{37}^{k'}$ and is given for reference.

Table 1

Representative examples of the changes in inflexion point (IP; Eq. (10)) and $U_{37}^{k'}$ indices measured by gas chromatography coupled to flame ionization detection (GC–FID $U_{37}^{k'}$) and positive ion chemical ionization mass spectrometry (GC–MS $U_{37}^{k'}$)

GC–FID $U_{37}^{k'}$	Total alkenones (ng)					
	200		100		50	
	IP	GC–MS $U_{37}^{k'}$	IP	GC–MS $U_{37}^{k'}$	IP	GC–MS $U_{37}^{k'}$
0.9	0.28	0.97	0.35	0.97	0.42	0.96
0.8	0.43	0.90	0.47	0.90	0.50	0.90
0.7	0.60	0.80	0.59	0.80	0.59	0.80
0.6	0.80	0.65	0.74	0.67	0.68	0.69
0.5	1.06	0.49	0.91	0.52	0.78	0.56
0.4	1.37	0.33	1.11	0.37	0.87	0.42
0.3	1.78	0.19	1.34	0.24	1.02	0.28
0.2	2.32	0.095	1.63	0.13	1.16	0.16
0.1	3.09	0.033	1.98	0.047	1.32	0.057

Comparison of the $U_{37}^{k'}$ expressions for GC–FID (Eq. (1)) and Eqs. (8) and (9) provides the following expression for GC–FID $U_{37}^{k'} = \text{GC–MS } U_{37}^{k'}$:

$$\text{IP} = (c_{3M}C_{37:3} + b_{3M}) / (c_{2M}C_{37:2} + b_{2M}) = 1 \quad (10)$$

Accordingly, at:

$$\text{IP} > 1 \quad \text{GC–FID } U_{37}^{k'} > \text{GC–MS } U_{37}^{k'}$$

and, at:

$$\text{IP} < 1 \quad \text{GC–FID } U_{37}^{k'} < \text{GC–MS } U_{37}^{k'}$$

The results corresponding to the examples summarized in Figs. 2 and 3 are shown in Table 1.

In general, at high $U_{37}^{k'}$, GC–FID $U_{37}^{k'} < \text{GC–MS } U_{37}^{k'}$, and at low $U_{37}^{k'}$, GC–FID $U_{37}^{k'} > \text{GC–MS } U_{37}^{k'}$.

3.4. Dependence on analyte amount

Further examples of the differences in quantitation afforded by GC–FID $U_{37}^{k'}$ and by GC–MS $U_{37}^{k'}$ are shown in Fig. 5 where the results obtained in the calculation of $U_{37}^{k'}$ for mixtures with the same $U_{37}^{k'}$ ratios but different alkenone concentrations are plotted. In agreement with the previous expressions, GC–FID $U_{37}^{k'}$ is lower than GC–MS $U_{37}^{k'}$ at $U_{37}^{k'}$ values of 0.5 or higher and GC–FID $U_{37}^{k'}$ is higher than GC–MS $U_{37}^{k'}$ at $U_{37}^{k'}$ values of 0.2 or lower. The differences in $U_{37}^{k'}$ between GC–FID $U_{37}^{k'}$ and GC–

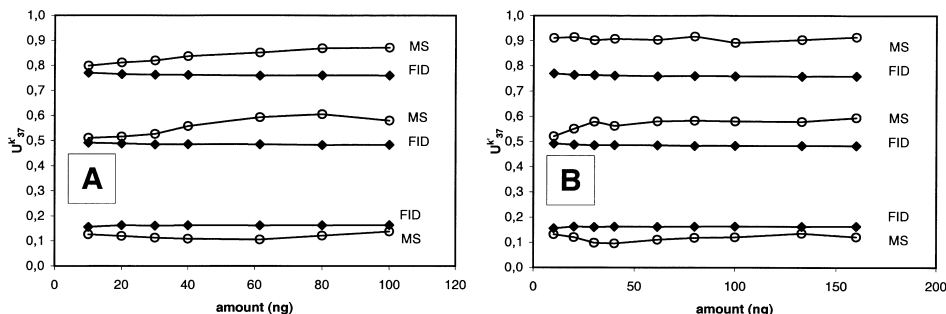


Fig. 5. Differences between $U_{37}^{k'}$ indices measured by GC coupled to FID and MS in the positive ion chemical ionization mode measured over a series of C_{37} standard mixtures at different concentrations. A and B panels refer to different instruments.

MS $U_{37}^{k'}$ are rather constant, independently of alkenone concentrations.

4. Conclusions

Non linear effects in the signal to amount of analyte ($S-C$) ratio of C_{37} alkenones analysed by GC-PCI-MS give rise to significant deviations between the $U_{37}^{k'}$ values measured with this technique and with GC-FID irrespective of any differences in sensitivity between $C_{37:2}$ and $C_{37:3}$. The main discrepancy in relation to previously observed results using linear calibrations concerns the changes in $U_{37}^{k'}$ values measured with GC-FID and GC-MS over the 0–1 range. Thus, at low $U_{37}^{k'}$ values (currently <0.3), the GC-MS determinations are consistently lower than those measured with GC-FID whereas at high $U_{37}^{k'}$ values (>0.4) they are higher. Calibration of the instrument with standards of known alkenone composition is recommended in order to maximize the linearity of the $S-C$ ratio.

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