Determination of alkenone unsaturation ratios by direct-high resolution mass spectrometry

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The degree of unsaturation in long chain (C_{37}) alken-2-ones (alkenones) (* Unsaturation is expressed as: $U^K_{37} = [C_{37:2}]/[C_{37:2}+C_{37:3}]$) biosynthesized by algae of the haptophyte order Isochrysidales has previously been shown to be correlated with growth temperature and exploited in palaeoceanography as a 'palaeothermometer' to study past changes in sea surface temperature (SST) (Brassell *et al.*, 1986). The conventional measurement typically involves extraction and isolation of an alkenone-containing fraction from the sediment prior to analysis by Gas Chromatography (GC) with flame ionization detection (FID). The GC-based method is both sensitive and precise. However, insufficient purification, or degradation of chromatographic performance can both result in the presence of co-eluting GC peaks which can significantly degrade the accuracy of the determined ratios (and hence SST estimates). Moreover, the speed of sample analysis is limited by the GC cycle time which is typically in excess of 1 hr.

In an attempt to overcome these analytical limitations we have investigated the potential of direct high resolution mass spectrometry (HRMS) to determine alkenone unsaturation ratios. Previous experiments using direct temperature-resolved mass spectrometry at nominal mass resolution indicated that thermal desorption of lipid extracts yielded similar information to that derived from GC-based analyses (Eglinton *et al.*, 1996).

Methodology

Total lipid extracts or alkenone-containing fractions (purified via silica gel chromatography) were introduced directly into a VG Autospec-Q mass spectrometer using a resistively heated platinum filament desorption probe. Samples were ionized at 12eV under EI conditions and analysed at 15,000 resolution in voltage scanning mode. Continuum data were acquired at 40,000 resolution, and integrated across time/temperature interval that the alkenones evolved. The areas of the 528.5271 ($C_{37:3}$) and 530.5427 ($C_{37:2}$) molecular ion masses were used to calculate the U^K_{37} ratios. GC measurements were performed on identical fractions using conventional conditions (Lehman *et al.* 1998).

Results

A suite of alkenone mixtures varying in degree of unsaturation was prepared from extensively purified fractions derived from a culture of *Isochrysis galbana*. Comparison of the GC-FID and HRMS data for the standards (Fig. 1) produces a simple, although slightly non-linear relationship. The exact values for the two analyses differ, probably due to differences in ionization efficiency or fragmentation of the two compounds. The HRMS-derived ratios are reproducible over time if care is taken in instrumental setup, and U^K_{37} values can be calibrated using standard mixtures with known ratios.

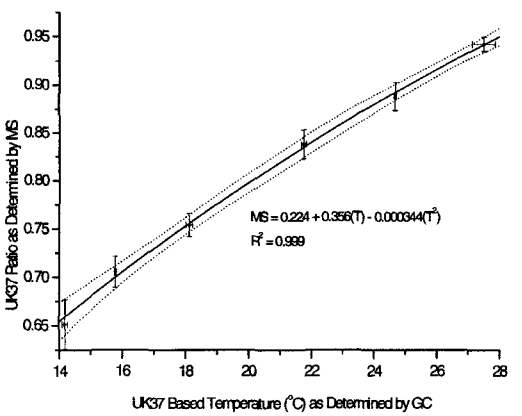

FIG. 1. Cross-plot of U^K_{37} determined by HRMS *vs* U^K_{37} derived temperature from GC for purified alkenones from *I. galbana* cultures. Dashed lines show 95% confidence interval.

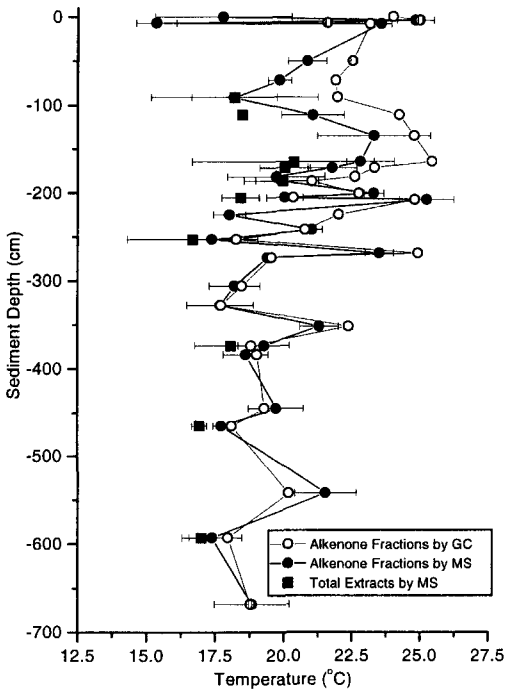

FIG. 2. Depth profiles of U^K_{37} derived temperatures determined by GC and MS for Bermuda rise core GPC-5. In addition to measurements of alkenone fractions selected MS measurements on total lipid extracts are shown. Temperatures are estimated based on the calibration of Prahl and Wakeham (1987).

Figure 2 shows GC- and HRMS-based depth profiles of SST for a sediment core spanning the last ~18,000 yr from the Bermuda Rise, northwest sub-tropical Atlantic ocean (Lehman *et al.* 1998). While the profiles generally parallel one another, particularly at deeper intervals (> 240 cm), significant discrepancies between the two measurements are evident for several samples. While the reasons for these differences are presently unclear, review of the GC data indicates the presence of partially co-eluting compounds that may have influenced the determined alkenone ratios. In comparison the HRMS data for these samples show well-resolved ions.

Interestingly, in all cases, the total lipid extracts analysed by HRMS (Fig. 2) yielded lower U^K_{37} values than the corresponding purified alkenone fractions. This may be due either to interference by other compounds at the masses selected (i.e. higher mass resolution is required), or some fractionation effect during subsequent sample work-up.

The direct MS technique is shown to be sufficiently precise ($\pm$ 1.0°C), equally sensitive to the GC measurement, and rapid (< 6 min./analysis). Further, the availability of visual inspection for interfering ions in continuum mode data allows for greater analytical certainty and even for the possibility of analysing crude, non-purified sediment extracts, or possibly even total particles (Eglinton *et al.*, 1996).

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References

Eglinton, T.I., Boon, J.J., Minor, E.C., and Olson, R.J. (1996) *Mar. Chem.*, **52**, 27–54.

Lehman, S.J., Eglinton, T.I., Keigwin, L.D. and Prell, W. (1998) *Paleoceanog.* In Prep.

Brassell, S.C., Eglinton, G., Marlowe, I.T., Pflaumann, U. and Sarnthein, M. (1986) *Nature*, **320**, 129–33.

Prahl, F.G. and Wakeham, S.G. (1987) *Nature*, **330**, 367–9.