Modelling of the Effect of Chromatographic Resolution on the Determination of the U^{K}_{37}' Index

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Abstract

The U^{K}_{37}' index is based on the measurement of the relative abundance of C_{37} alkenones in marine samples and is used to estimate absolute palaeo-sea surface temperatures. The reliability of the data (and the value of their interpretation) depends on the minimization of those factors that give rise to errors in the measurement of U^{K}_{37}'. The index is calculated using gas chromatography, and here, the effect of chromatographic resolution on U^{K}_{37}' is evaluated through the modelling of chromatograms with Gaussian or exponentially modified Gaussian peaks. It is shown that chromatographic resolution of the alkenones is indeed linked to the accuracy of U^{K}_{37}', although the magnitude of the error depends on the actual values of U^{K}_{37}', the degree of peak tailing, and the relative abundance of coelutants. However, it is not necessary to achieve baseline separation between the alkenones to obtain U^{K}_{37}' values with errors that are climatically nonsignificant. Further work should demonstrate if these results can be reproduced experimentally, but in principle, the use of partially unresolved chromatograms with or without peak tailing does not appear to be a major source of error during U^{K}_{37}' analysis.

Introduction

The C_{37} alkenones and the related alkyl alkenoates (Table I) are components found ubiquitously in the marine environment and are biosynthesized by several species of phytoplankton. Hence, in organic geochemical terms, they can be considered chemical fossils and biomarkers for algae of the class *Prymnesiophyceae* (1,2). In the early 1980s, a linear relationship between the relative abundance of alkenones and the temperature of the media where they were biosynthesized was demonstrated both in laboratory cultures and the open sea (3). Further studies gave a numerical expression to this relationship with the definition of the U^{K}_{37} index and its simplification, U^{K}_{37}' (4–6):

$$U^{K}_{37}' = [37:2Me]/([37:2Me] + [37:3Me]) \quad \text{Eq. 1}$$

The U^{K}_{37}' index has quickly gained acceptance as a paleoclimatic proxy, because it provides a direct and relatively easy way to reconstruct sea surface palaeotemperatures for the Quaternary (a compilation of sediment data in Reference 7). To date, all the analytical methods used to determine U^{K}_{37}' have relied on high-resolution gas chromatography (GC) to isolate the alkenones from complex natural mixtures and calculate their relative abundance. Normally, baseline peak separation (i.e., complete resolution) of the components is considered to be necessary to measure the peak areas with enough accuracy so that palaeotemperature estimates are not biased because of peak overlapping, although this has not been demonstrated. During the analysis of U^{K}_{37}' in locations from higher latitudes (e.g., from 50°N in the North Atlantic), the chromatograms are further complicated by the increasing abundance towards the colder sites of the alkyl alkenoates. Thus, longer analysis times are needed to achieve baseline peak separation, and analysis times can be as long as 80 min per sample. This poses a problem because, to obtain sufficient data for climatic interpretation, large numbers of samples have to be analyzed, emphasizing the need for a rapid and reliable analytical procedure to obtain reliable U^{K}_{37}' data. The latter is particularly important, because temperature records are interpreted in terms of the absolute and relative magnitude of the estimates; thus, data are compared with those derived from other laboratories and estimates derived from other proxy approaches.

Table I. Components Discussed in the Text

Shorthand notation	Name
37:3 Me	heptatriaconta-8E,15E,22E-trien-2-one
37:2 Me	heptatriaconta-15E,22E-dien-2-one
36:3 OMe	methyl hexatriaconta-7E,14E,21E-trienoate
36:2 OMe	methyl hexatriaconta-14E,21E-dienoate

Hence, it is necessary to evaluate all the factors that may give rise to biases in the U^{K}_{37}' data.

In this paper, the theoretical consequences of reduced chromatographic resolution on the accurate determination of the U^{K}_{37}' index is discussed. The calculations are based in the proviso that chromatographic peaks approximate a Gaussian curve if there is no tailing or an exponentially modified Gaussian (EMG) curve if tailing occurs, and that there is no coelution of any other component with the alkenones and alkyl alkenoates. The model calculations carried out are partly based on previous works concerning sources of error in the determination of chromatographic peak size ratios (8,9) and the quantitative errors of individual peaks as a function of resolution (10,11), which partly follows up on the early work of Snyder (12). In this study, two cases ($U^{K}_{37}' > 0.55$ and $U^{K}_{37}' < 0.60$) are considered. In the former case, only the di- and triunsaturated C_{37} alkenones are considered in the calculations (the two-peaks case, Figure 1), whereas for the latter, the presence of alkyl alkenonates in the elution region of the alkenones (the four-peaks case, Figure 2) has also been taken into consideration.

Experimental

Modelling of the chromatographic peaks and other mathematical calculations (integration, derivation, equation solving, etc.) were carried out using the Mathcad software package (Mathsoft, Cambridge, MA). The simulation of the detector signal $G(t)$ was equivalent to the sum of 4 normalized Gaussian functions $G_i(t)$, corresponding to up to 4 peaks (i = 1 to 4).

$$G_i(t) = m_i / [\sigma_i(2\pi)^{1/2}] \exp[-(t_i - t)^2/2\sigma_i^2] \qquad \text{Eq. 2}$$

This function was evaluated for increments of t = 0.001, which is equivalent to a data acquisition sampling rate of

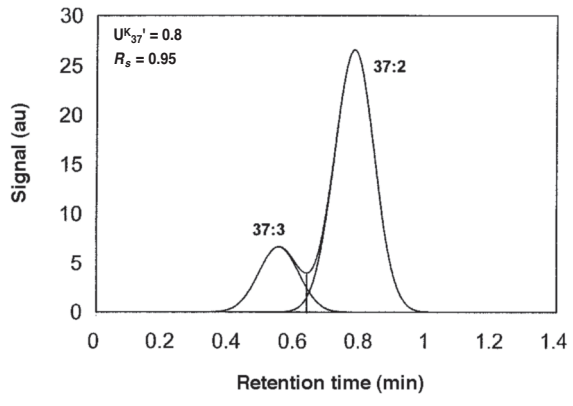

Figure 1. Computer simulated chromatogram for the C_{37} alkenones (triunsaturated, 37:3; diunsaturated, 37:2) with the individual peak curves and the overall signal curve (i.e., detector signal, ΣG_i). The vertical drop line from the valley in the overall signal curve shows the integration boundary between peaks.

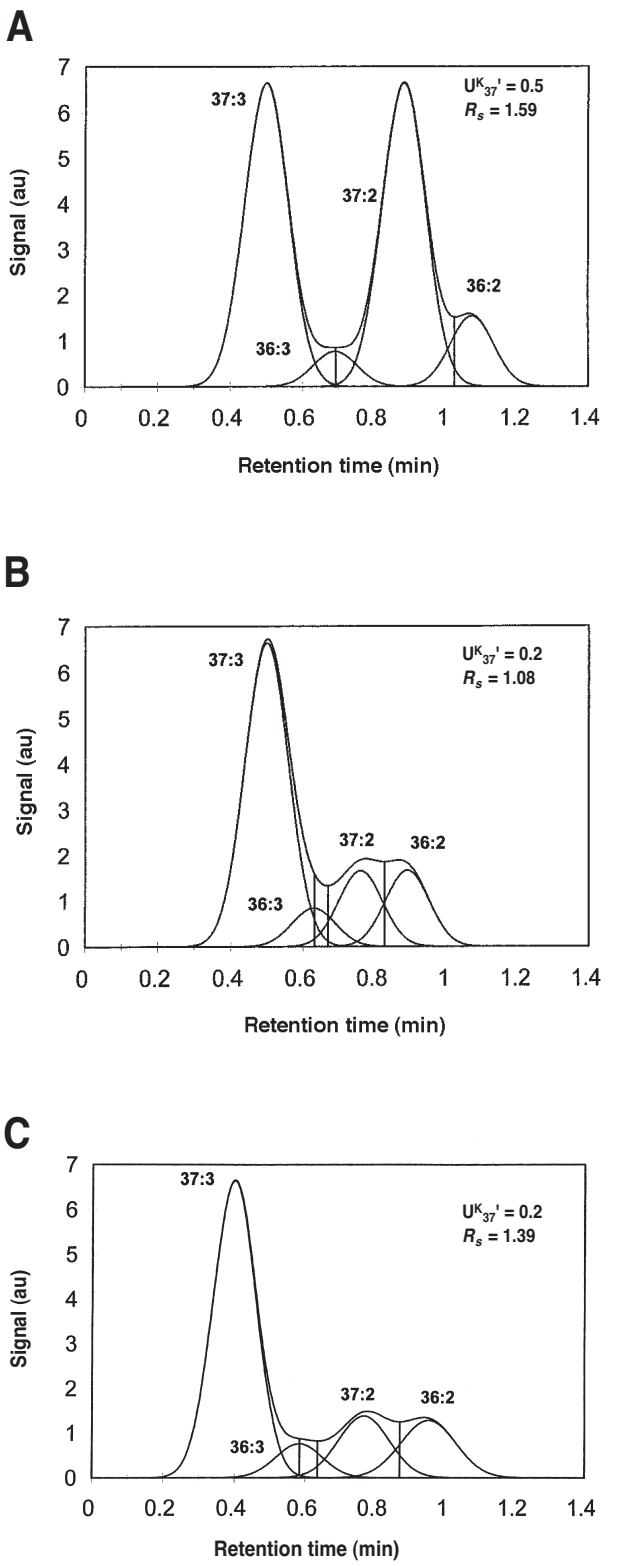

Figure 2. Computer simulated chromatogram of the C_{37} alkenones and the C_{36} alkyl alkenoates (triunsaturated, 36:3; diunsaturated, 36:2) with the individual peak curves and the overall signal curve (i.e., detector signal, ΣG_i): $\sigma_1 = \sigma_2$ (A,B) and $\sigma_{i+1} = 1.1\sigma_i$ (C). The vertical line at the 36:3 alkenoate retention time marks the 37:3 alkenone integration limit, and the vertical lines to the left and right of the 37:2 alkenone peak correspond to the component integration limit. The labeling of the figures relates to cases discussed in the text.

16.67 Hz in a GC system. For each chromatographic peak, the adjustable parameters were the standard deviation of the Gaussian distribution (σ_i, proportional to peak width), retention time (t_i), and analyte amount (m_i). This last parameter was varied to select the values of U^{K}_{37}' and the presence of alkenoates in the chromatographic trace. As an initial simplification, σ_i was considered constant for all peaks. This is clearly not always the case (e.g., as σ increases for the later eluting peaks, especially in isothermal conditions, we have observed it can be up to 10% larger for the diunsaturated versus triunsaturated alkenone), and this is discussed where appropriate in the text. A final assumption was the equalization of the 3 retention time increments between the 4 peaks (alkenones and alkyl alkenoates), which can be easily reproduced experimentally.

The EMG function is widely used to simulate non-Gaussian tailed peaks (13).

$$h_i(t) = (m_i/2\tau)\exp[1/2(\sigma/\tau - (t - t_i)/\tau)] \{\text{erf}[1/\sqrt{2}\,(t_i/\sigma - \sigma/\tau)] + \text{erf}[1/\sqrt{2}(\{t - t_i\}/\sigma - \sigma/\tau)]\} \quad \text{Eq. 3}$$

The simulation of the detector signal for the two alkenone tailing peaks was performed by the sum of two of these functions, where $h_i(t)$ is the equivalent of $G_i(t)$ in the Gaussian case, and τ is the time constant of the exponential component of $h_i(t)$. The parameters m_i, σ, and t_i are defined as in the Gaussian case.

The chromatographic peaks were integrated using the vertical drop method and a Romberg algorithm. The valleys between 2 peaks were found by solving the derivative of the peak generating functions using the secant method. The second derivative was employed if the vertical drop point could not be found using the first derivative, as it occurs when dealing with 4 peaks (alkenones and alkenoates) at low resolution, which leads to shouldering. If the peak overlap was so severe that the peak presence (e.g., 36:3 alkenoate) could only be inferred by a relatively flat valley between the alkenone peaks above the baseline, the vertical drop point was set at the retention time corresponding to the mean of the two alkenones' retention times.

While the two-peaks case is relatively simple to model (Figure 1), the four-peaks case is substantially more complex (Figure 2), because the abundance of alkyl alkenoates to alkenones changes with values of U^{K}_{37}', or rather, temperature (3,14,15). This variation has been constrained using data from natural samples (15).

$$\text{Total alkenoate amount (ng/g)} = 0.386 - 0.427\ U^{K}_{37}' \quad \text{Eq. 4}$$

A further simplification was to keep the ratio of the 2 alkenoates constant (twice as much diunsaturated as triunsaturated), which approximates the relationship found in natural data (16). These simplifications reduce the four-peaks case to a variation of the two-peaks case.

Resolution has been calculated on the simulated chromatogram according to the following formula:

$$R_s = 2.354(t_2 - t_1)/2(w_1 + w_2) \quad \text{Eq. 5}$$

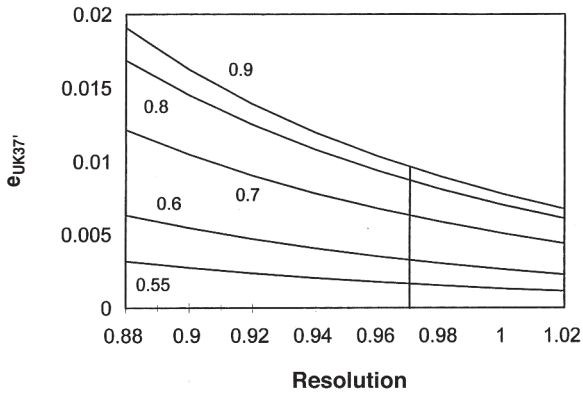

Figure 3. Influence of resolution (R_s) on U^{K}_{37}' error ($e_{UK37'}$) for several index values and $\sigma_1 = \sigma_2$. The error is the difference between the theoretical index value for 2 completely baseline resolved peaks and the actual value obtained from overlapped peaks. The vertical line indicates the minimum resolution required to obtain U^{K}_{37}' with acceptable errors.

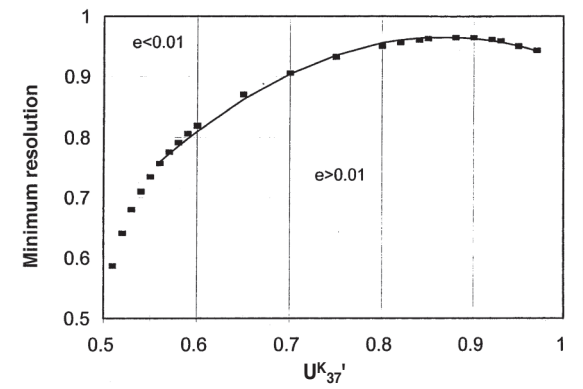

Figure 4. Plot of resolution (R_s) versus U^{K}_{37}' when $e_{UK37'} = 0.01$, $\sigma_1 = \sigma_2$, and only the alkenones are present in the chromatogram (as in Figure 1). The solid line is the quadratic curve fitted to the model points in the 0.55–0.97 range.

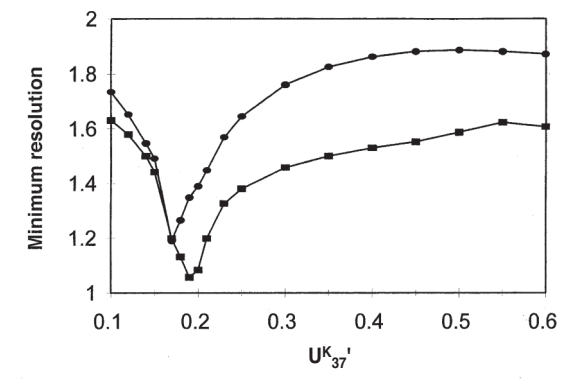

Figure 5. Plot of resolution (R_s) versus U^{K}_{37}' for $e_{UK37'} = 0.01$ when both alkenones and alkyl alkenoates are present in the chromatogram (as in Figure 2). The lower curve (n) corresponds to $\sigma_{i+1} = \sigma_i$, whereas the upper curve (⇑) corresponds to $\sigma_{i+1} = 1.1\sigma_i$.

where the superindexes 1 and 2 refer to the triunsaturated and diunsaturated alkenones, respectively, and peak width w is measured at the half-height of the peak.

Results and Discussion

Most of the results of the calculations are discussed in terms of the absolute value of the error of U^K_{37}' ($e_{UK37'}$). This has been calculated as the difference between U^K_{37}' measured from the prescribed values of m_i ($R_s = \infty$) and U^K_{37}' at a given resolution from the values of the integration of the components, I_i:

$$e_{UK37'} = [I_2/(I_1 + I_2)] - [m_2/(m_1 + m_2)] \quad \text{Eq. 6}$$

In relation to the use of U^K_{37}' as a climatic proxy, we consider that the resolution obtained during analysis should be that which allowed $e_{UK37'}$ not to be larger than 0.01, which approximates 0.3°C (according to the calibration equation)(17). This value of $e_{UK37'}$ is set arbitrarily, with the only consideration being that a larger error would be similar to the natural variability of U^K_{37}' (7).

The two-peaks case: alkenones with no tailing

The graph in Figure 3 shows $e_{UK37'}$ versus R_s for U^K_{37}' values ranging from 0.55 to 0.9. In agreement with previous studies (8,12), as the relative abundance between 2 peaks increases, the sensitivity to changes in resolution also increases. In other words, for increasing values of U^K_{37}', $e_{UK37'}$ is increasingly sensitive to resolution. For $U^K_{37}' = 0.5$, the error is always zero due to the compensation of the areas between both peaks, whereas for $U^K_{37}' = 0.9$, for instance, the resolution has to be approximately 1 so that $e_{UK37'} < 0.01$. When $U^K_{37}' < 0.5$, the curves in Figure 2 would mirror those in the upper range, but the absolute errors would be negative.

The main implication of Figure 3 is that, during GC analysis, if peaks are Gaussian, the overlap between peaks can be considerable before $e_{UK37'}$ is significant; thus, baseline peak separation of the alkenones is not required to derive acceptable U^K_{37}' values (i.e., $e_{UK37'} < 0.01$). As an example, the simulated chromatogram in Figure 1 for $U^K_{37}' = 0.8$, the resolution is set at the minimum resolution ($R_s = 0.951$), so $e_{UK37'} = 0.01$. Hence, during the GC analysis of a sample with $U^K_{37}' = 0.8$, if $R_s > 1.5$ (which is what most analysts would deem necessary), there could be the possibility of reducing analysis time by decreasing the resolution of the analysis before exceeding the maximum $e_{UK37'}$ of 0.01. This could be achieved by modifying the GC parameters (for example, using shorter and thus cheaper GC columns).

The plot in Figure 4 that shows values of R_s versus U^K_{37}' when $e_{UK37'} = 0.01$ also demonstrates that, in principle, good chromatographic resolution is not a critical parameter in deriving U^K_{37}' with palaeoclimatologically acceptable errors. The U^K_{37}'–R_s curve shows an increasingly steep slope as the index approaches 0.5, so severe peak overlap in this region should not prevent the determination of U^K_{37}' (e.g., $U^K_{37}' = 0.55$, minimum R_s of 0.735; Figure 1). The minimum resolution curve reaches a maximum near $U^K_{37}' \sim 0.9$ (or 0.1 when $U^K_{37}' < 0.5$), and then decreases again. This maximum corresponds to a resolution of approximately 0.97 (compared with a baseline separation of $R_s = 1.5$), and it represents the minimum theoretical R_s needed to analyze samples in the entire range of U^K_{37}' ($0.1 < U^K_{37}' < 0.9$) for $e_{UK37'} \leq 0.01$. For values of $U^K_{37}' < 0.9$, when $R_s = 0.97$, $e_{UK37'}$ is smaller than 0.01 (e.g., $e_{UK37'} = 0.003$ for $U^K_{37}' = 0.6$). A quadratic curve can be fitted to the data in Figure 4 in the 0.55–0.9 U^K_{37}' range:

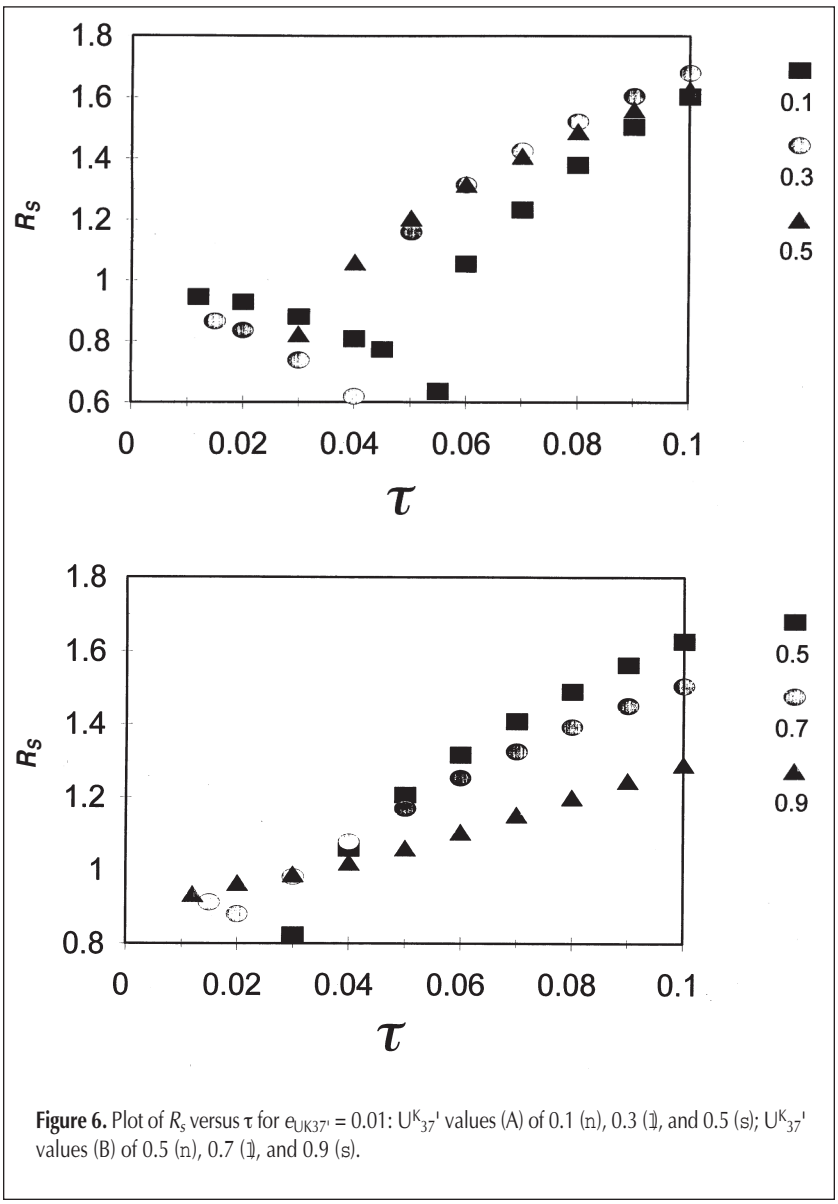

Figure 6. Plot of R_s versus τ for $e_{UK37'} = 0.01$: U^K_{37}' values (A) of 0.1 (n), 0.3 (l), and 0.5 (s); U^K_{37}' values (B) of 0.5 (n), 0.7 (l), and 0.9 (s).

$$R_{s,0.01} = -2.176(U^{K}_{37}')^2 + 3.774U^{K}_{37}' - 0.671 \quad \text{Eq. 7}$$

This provides a numerical expression of the safe theoretical resolution for a specific U^{K}_{37}' value if peaks are Gaussian. An experimental resolution smaller than the calculated one implies an error greater than the target of 0.01.

Incidentally, thus far we have considered that σ values, or the peak widths w of the alkenones, are equal. This is not usually the case during the analysis, because σ will increase with retention time. In this case, a correction factor should be considered to obtain values of resolution.

$$R'_s = R_s(\sigma_2/\sigma_1) = R_s(w_2/w_1) \quad \text{Eq. 8}$$

The four-peaks case: alkenones and alkenoates with no tailing

The presence of additional components in the chromatogram (alkenoates; Figure 2) significantly alters the optimum GC conditions for the determination of U^{K}_{37}', as previously described. Consideration of the importance of the value of σ is also more complex, and the simulations have been carried out for 2 cases: equal σ for all components and $\sigma_{i+1} = 1.1\sigma_i$. The latter is proposed as the worst case scenario. In fact, during real GC analysis, the components will be found to have similar σ values, because the retention times of the components are within a few minutes of each other, and substantial peak broadening does not occur. Furthermore, GC conditions (e.g., using adequate temperature gradients instead of isothermal conditions) can also be optimized so that all σ_i values are equal.

The minimum R_s-versus-U^{K}_{37}' curves for the four-peaks case is shown in Figure 5. As in the previous section, the maximum $e_{UK37'}$ is set at 0.01, and the value of resolution discussed in the text and shown in the graph is that calculated between the 2 alkenone peaks. The 2 curves in Figure 5 for equal and different σ values are very similar (although offset from each other). Their interpretation is certainly more complicated than in the two-peak case curve in Figure 4 due to the complex interplay between the 4 components, their relative abundance (as a function of U^{K}_{37}'), and the extent of overlapping (function of R_s). In Figure 5, the minimum resolution curve falls sharply to a minimum of R_s at $1.05_{\text{same s}}$ or $1.2_{\text{different s}}$ when $U^{K}_{37}' = 0.18_{\text{same s}}$ or $0.16_{\text{different s}}$ and contains 2 maxima for values of U^{K}_{37}' at 0.1 and $0.5_{\text{different s}}$, or $0.55_{\text{same s}}$, respectively.

In the region where the minimum of the curve occurs, the peak sizes of the 36:2 alkenoate and the 37:2 alkenone are close, so that the errors incurred in the integration of the peak areas of the 2 components compensate each other to a similar extent (Figures 2B and 2C). As peak sizes become increasingly different, the minimum resolution curve rises again sharply between U^{K}_{37}' ~ 0.1–0.2 and 0.2–0.3 and with a lower slope between U^{K}_{37}' ~ 0.3–0.55 as the amount of alkenoates decreases. Finally, for $U^{K}_{37}' > 0.6$ and equal peak σ, the chromatogram turns gradually into the two-peaks case, and the required minimum R_s steeply decreases. In contrast, when σ is different for $U^{K}_{37}' > 0.6$, despite the much higher and increasing relative abundance of the alkenones to alkenoates, these components still have a significant weight in the calculation of U^{K}_{37}', so R_s needs to be kept relatively high to minimize $e_{UK37'}$.

The values of R_s in Figure 5 are higher than those in Figure 4 for the same U^{K}_{37}' values, but baseline separation of the 4 peaks (i.e., $R_s = 3$) is not required to derive U^{K}_{37}' with acceptable errors. This is illustrated in the example in Figures 2A and 2B, where the 36:3 alkenoate peak cannot be easily distinguished in the simulated GC chromatogram. An experienced chromatographer would probably recognize the presence of a component between the alkenones as an anomalous overlap between the 2 alkenone peaks, because the alkenone peaks would otherwise be almost baseline resolved. However, the drawing of the integration drop lines would split up the alkenoate peak, and its area would be included with those of the alkenones.

As in the two-peak cases, a theoretical minimum resolution can also be obtained to calculate U^{K}_{37}' with an acceptable error. Thus, if alkenoates are present in the chromatogram, GC conditions could be optimized to $R_s = 1.62$, if σ values are equal (e.g., Figure 2B) and the peaks are Gaussian. A very cautious option is to assume that σ can vary substantially (e.g., Figure 2C); then, the minimum working R_s for any value of U^{K}_{37}' is 1.89, which is still a substantially lower R_s value than if baseline peak separation is obtained between the 4 components (i.e., $R_s = 3$). In fact, most analyses will have a peak σ value between the 2 scenarios considered, and the theoretical optimum R_s can be obtained by interpolating between the curves in Figure 5.

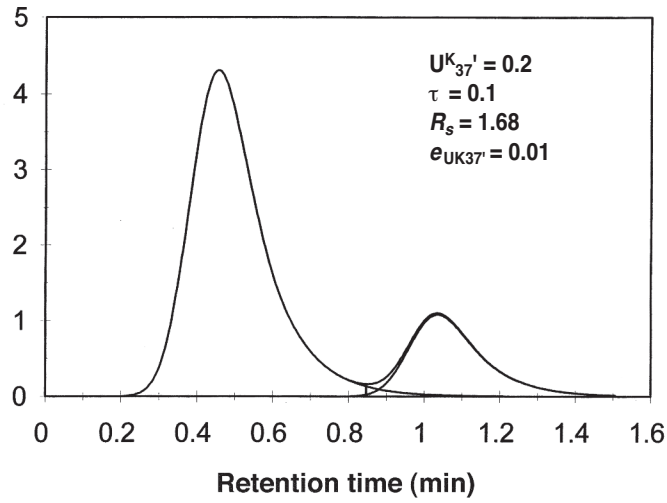

Figure 7. Computer simulated chromatogram for the C_{37} alkenones (left peak, triunsaturated; right peak, diunsaturated) with the individual EMG peak curves and the overall signal curve (i.e., detector signal, Σh_i). The vertical drop line from the valley in the overall signal curve shows the integration boundary between peaks.

Effect of tailing

So far, we have discussed the effect of chromatographic resolution on $e_{UK37'}$ in the case of Gaussian peaks, which are symmetrical, and therefore represented an idealized situation. In reality, peaks in a chromatogram often have a degree of tailing. If it is strong, however, it is a symptom of bad chromatographic conditions (e.g., dead volume, a dirty column with active sites, etc.) and therefore should be corrected or reduced to minimal levels. To illustrate the effects of tailing on resolution, the peaks were modelled using an EGM function, and the values of R_s were plotted when $e_{UK37'} = 0.01$ at different U^K_{37}' and τ values from 0 to 0.1, corresponding to a factor of asymmetry (calculated at 10% of peak maximum height) between 1 and ~1.8 (Figure 6).

In general, when tailing (i.e., τ) increases, a higher resolution is required to obtain U^K_{37}' with $e_{UK37'} < 0.01$ (Figure 6), except in the case of $U^K_{37}' = 0.1$ or 0.3, and $\tau < 0.05$ (Figure 6A). Then, the trend is inverted as the peak areas compensate in such a way that for increasing τ, resolution can be decreased to achieve an acceptable $e_{UK37'}$ (note that in this range of τ, the error is negative). The higher the value of U^K_{37}', the less sensitive R_s is to variations in τ. As in the case of Gaussian peaks, it is not necessary (in principle) to achieve baseline separation between peaks to obtain U^K_{37}' with an acceptable error, which is illustrated by the modelled chromatogram in Figure 7. Nonetheless, for a given U^K_{37}' value with $e_{UK37'} < 0.01$, if tailing occurs, resolution has to be higher than if the peaks are only Gaussian.

Conclusion

The importance of resolution on the determination of U^K_{37}' was evaluated. These modelling experiments confirm that the accurate measurement of the index by GC is dependent on the alkenones' chromatographic resolution, the extent of peak tailing, and the relative abundance of coeluting components (e.g., alkyl alkenoates). However, in principle, it may not be necessary to reach baseline separation between the alkenones to obtain U^K_{37}' values with errors climatically insignificant ($e_{UK37'} < 0.01$). This applies to all the cases studied, although the theoretical minimum resolution needed to obtain reliable data depends on the values of U^K_{37}'. Moreover, the resolution has to increase substantially depending on the presence of tailing and coeluting components with the alkenone peaks. Further work should demonstrate if our results can be reproduced experimentally, but we have shown that low chromatographic resolution by itself (for instance, if there is a small coelution with the alkenones) is not likely to be a major source of error during U^K_{37}' analysis.

Acknowledgments

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