Computer treatment of gas–liquid chromatographic data, with special reference to fatty acid methyl esters

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ABSTRACT A computer program is described which analyzes output punched directly onto paper tape from a gas–liquid chromatograph. Although this program was written specifically for samples of fatty acid methyl esters derived from adipose tissue triglycerides which are eluted within 1 hr, modification of the dimension statements in the program would enable it to deal with samples which require a longer time to come off the column.

The salient features of the rationale of the program are discussed in detail, particularly the procedures for base line correction and for estimating the contributions from components which are not perfectly separated in the column. Examples are given of the program in practice, of comparing the results it gives with those obtained by manual triangulation of the areas on a recorder chart, and of indicating the range of column load over which we have found that it operates satisfactorily. A sample computer print-out from the program is presented and interpreted.

SUPPLEMENTARY KEY WORDS carbon number · methyl palmitate · methyl stearate · triangulation · adipose tissue

FOR SEVERAL YEARS following the advent of GLC as a tool for the analysis of mixtures of fatty acid esters, the measurement of peak areas and relative retention times and the derivation of the carbon number of each component, were rather tedious procedures. Although the introduction of integrating devices of various kinds made the task somewhat less onerous, their limitations, such as failure to resolve the components of overlapping peaks, led us to investigate the possibility that direct treatment by a computer of raw data could provide a reliable and more rapid analysis of gas chromatograms. Other methods have been reported in the literature, but they appeared to us to have disadvantages in routine use; these we will discuss later in this paper. The computer program to be described is tailored to the output of a particular GLC system, but the principles involved are generally applicable to other detection systems.

METHODS

The amplified output from the ^{90}Sr detector of an argon gas chromatograph (Pye Unicam Ltd., Cambridge, England) was simultaneously recorded by both a conventional potentiometric recorder and a data-logging system comprising a digital voltmeter and scanner (Solartron Electronic Group Ltd., Farnborough, England) with a drive to a paper-tape punch (Addo Ltd., Hatfield, England). This output, in millivolts, is directly proportional to the ionization current developed in the detector and, consequently, is directly proportional to the mass of ester present, as noted by Lovelock, James, and Piper (1). Under conditions where this linear relationship does not obtain, the program would simply require one additional Fortran statement in order to carry out the necessary linearizing transformation. The paper tape was punched at approximately 2-sec intervals according to paper-tape code PTTC/8 HEX, the code compatible with the IBM 1130 computer. For GLC 4-ft-long glass columns (I.D., 4 mm) were packed with acid-washed Celite 545, 85–100 mesh (Shandon Scientific Co., London, England) impregnated with either 15% of

Annotated listings of the program may be obtained from Mr. A. W. Boyne at a cost of 5s. (25 np) or $.60 (US) to meet the cost of production and postage.

Abbreviations: GLC, gas–liquid chromatography.

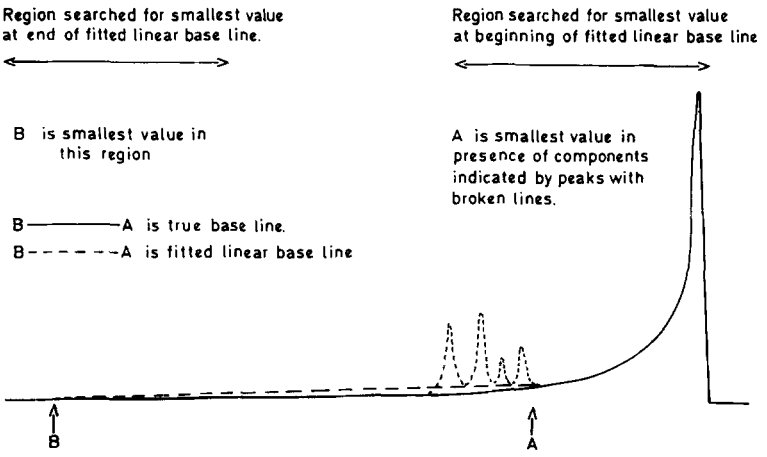

FIG. 1. Diagrammatic representation of situation in which estimated linear base line differs from true base line. As point *A* is forced to the right, the discrepancy between true and linear base line increases.

polymerized ethylene glycol adipate (PEGA) or 7% Apiezon L grease. The operating temperatures were 180°C and 185°C for the PEGA and Apiezon columns, respectively, and the argon gas flow was 55 cc/min. A consequence of these conditions is that the entire sample passed through the detector within 1 hr so that the array of signals given at 2-sec intervals consisted of not more than 1800 values. This limitation is specified by a dimension statement in the programs described here; in applications involving larger arrays, this dimension would have to be increased. The data from the chromatograph was prefaced by information entered by a manual entry unit, giving the identity of the sample analyzed and indicating whether the subsequent data emanated from an Apiezon or PEGA column.

The program has been used to determine the fatty acid composition of about 300 samples of adipose tissue triglycerides of several animal species. In these samples only very small proportions of unsaturated C_{20} components were present, and these were determined by analysis of a hydrogenated sample in which they appeared as 20:0[1] which was eluted within 1 hr.

FEATURES OF THE PROGRAM

The following suite of programs described here (*a*) reads and smoothes the data, (*b*) detects the onset of the peak associated with the solvent, (*c*) corrects the values of the array for base line, (*d*) detects and locates the number of peak complexes with one, two, three, or four maxima, (*e*) estimates the number of components in each peak complex, (*f*) estimates the area attributable to each component in each peak complex where this is possible, (*g*) identifies the carbon number associated with each component, and (*h*) prints out an analysis of the sample indicating for each component its distance along the recorder chart from the onset of the solvent peak.

When the data have been read and smoothed, the onset of the solvent peak is very easily detected, and this is the point from which the elution times of all the components are measured. The base line is estimated by linear interpolation between the smallest value in the first 200 points after the solvent peak and the smallest value in the last 300 in the array, which consists of about 1800 points in all. The base line value is then subtracted from the value of each point in the array. In practice the base line is very nearly linear, but not exactly so, possibly because the concentration of solvent in the column approaches zero asympototically with time. Fig. 1 illustrates, in exaggerated form, the difference between the true base line and that estimated from the smallest values (*A* and *B*) at either end of the array. Where precisely these points *A* and *B* lie along the array depends upon the esters present in any given sample.

Because of this nonlinearity of the true base line, the result of subtraction of the estimated linear base line is that peak complexes in the central region of the array start and end with small negative values. Although this is of little consequence if the peak area is large, it may lead to appreciable underestimation of the areas of small peaks if only the area above the linear base line is considered. Therefore, as standard procedure, if a peak complex starts from a negative value, the area of the complex is estimated by subtracting the first value from each value in the peak complex. In other words, the total area above the true base line is calculated.

After the linear base line correction has been made, the array is searched for peak complexes with up to four maxima. There is no reason why more than four maxima

[1] Fatty acids are designated in the Tables and, as appropriate, in the text according to the shorthand nomenclature of Dole, James, Webb, Rizack, and Sturman (2).

should not be considered, but so far we have found four to be adequate for all the samples we have studied. As a result of this examination, information is stored of the number of peak complexes with a single maximum, along with the location of the onset, the maximum and the end of each peak, as well as the area of the complex estimated as indicated above. For peak complexes with more than one maximum, the position of each trough and maximum is also stored.

Although the data have been smoothed, it is still possible for small spikes, possibly due to electronic noise, to remain in modified form in the array, so the program rejects peaks of less than 16 sec in duration. This is achieved by insuring that at least four successive values are greater than the one preceding it, and that four similarly decrease before accepting an area as a genuine peak. Another criterion applied in this program is that the value of a maximum should be at least 0.1 mv greater than the base value from which it rises (full-scale deflection is about 17.0 mv, a limitation imposed by the detector).

The next part of the program examines the shape of each peak complex. This is done by an examination of the second derivative and is illustrated in Fig. 2, where two double-peaked complexes are shown, I with two components, and II with three components. IA and IIA represent the response curves, IB and IIB, the slopes of the response curves (the first derivative), and IC and IIC, the slopes of the first derivatives (the second derivatives). The second derivative associated with peak complex I changes sign four times; that associated with peak complex II changes sign six times. The estimated number of components in a peak complex is obtained by halving the number of sign changes in its second derivative. Because the data have already been smoothed and because it is only the sign of the second derivative in which we are interested, the quantities examined by the program are the differences between successive pairs of values in the smoothed array i.e. $x_{j+1} - 2x_j + x_{j-1}$.

Information is also retained where these sign changes occur in relation to the position of the maxima. In complex II, for example, three sign changes take place to the right of the first (or right-hand) peak, so we know that the additional component, if it is such, occurs on the leading edge of the peak, and its position can be given as approximately halfway between the first and second sign-changes in the second derivative.

Provided that the number of components exceeds the number of maxima by no more than one, and provided also that the minor component is on a leading or trailing edge of the complex, it is possible to estimate the area attributable to each component. If both these conditions are not met, the program prints a message indicating the number of possible components in the complex and, in addition, gives the area attributable to each of the main components (i.e. those components associated with the maxima) on the basis of a simple trough to trough summation. That the estimate has been done in this way is indicated in the printed output.

Estimation of area for single-peak complexes with one component is achieved by accumulation of the area under the curve. For other complexes in which the num-

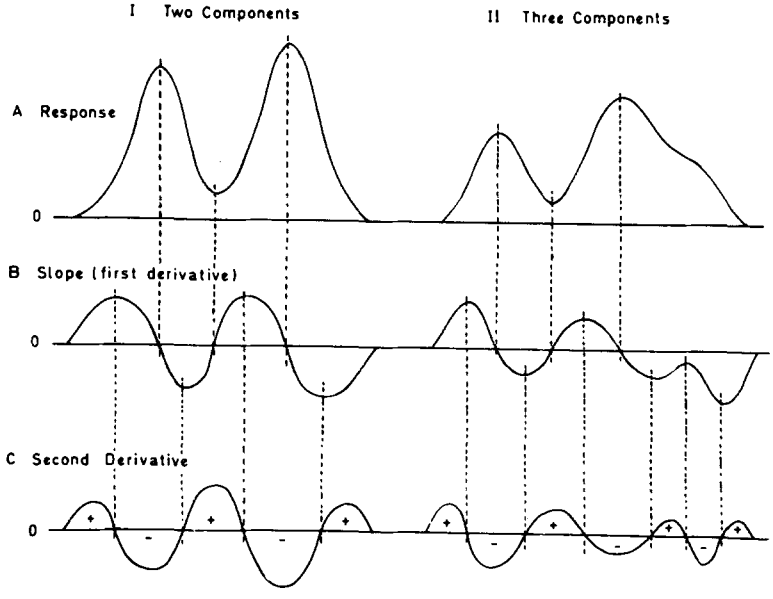

FIG. 2. Illustration of method of estimating number of components in a peak complex. Vertical dotted lines indicate that maxima and minima of response, *A*, coincide with zeros of slope, *B*; similarly maxima and minima of slope, *B*, coincide with zeros of second derivative, *C*. The number of sign changes in the second derivative is twice the number of components.

ber of components equals the number of maxima, the area attributed to each is based on the assumption that responses to adjacent components are similar in shape. The aspect of shape utilized is the proportion of the total area which lies before the maximum. Fig. 3 illustrates the basis of the method for a two-component doublet.

In Fig. 3 the two components are I and II. The onset of the complex is at M, the maxima at N and P and the tail is at R. If the areas of components I and II are A and B respectively, the total area, which is measured, is $A + B$.

The shaded portion of component I up to its maximum is A_1, (above MN), so $A = FA_1$ where F has to be estimated.

The area above NP is $A_2 + B_1$ where $A_2 + A_1 = A$. $A_2 + B_1$ is denoted by S_1.

$$\begin{aligned} \text{Then } B &= (A_1 + S_1 - A)F \\ &= (A_1 + S_1 - A_1F)F \\ T(=\text{Total area}) &= A + B \\ &= A_1F + (A_1 + S_1 - A_1F)F \\ &= (2A_1 + S_1)F - A_1F^2 \end{aligned}$$

If we estimate F, the difference between the measured and the estimated total area is

$$E = T - (2A_1 + S_1)F + A_1F^2$$

and the estimate of F may be obtained by minimizing E^2.

We noted that F was always near to 2.27, so an iterative procedure has been used, replacing F by $D + X$, and taking $D = 2.27$ for the first iterative cycle:

$$\begin{aligned} E &= T - (2A_1 + S_1)D + A_1D^2 - \\ &\quad (2A_1 + S_1 - 2A_1D)X + A_1X^2 \\ &= \alpha + \beta X + \gamma X^2 \\ E^2 &= (\alpha + \beta X + \gamma X^2)^2 \\ \frac{d(E^2)}{dX} &= 2(\alpha + \beta X + \gamma X^2)(\beta + 2\gamma X) \end{aligned}$$

This expression is equated to zero, ignoring terms in the second and higher powers of X, since X is small. This gives:

$$\alpha\beta + (\beta^2 + 2\alpha\gamma)X = 0$$

where

$$\begin{aligned} \alpha &= T - (2A_1 + S_1)D + A_1D^2 \\ \beta &= -(2A_1 + S_1 - 2A_1D) \\ \gamma &= A_1 \end{aligned}$$

This equation is solved for X, and the estimate obtained for X is put into the equation for A and B,

$$\begin{aligned} \text{Thus} \quad & A = A_1(D + X) \\ \text{and} \quad & B = [A_1 + S_1 - A(D + X)][D + X] \end{aligned}$$

If the sum of these is within 0.5% of T, these values of A and B are accepted by the program; if not, then D is increased by the estimate of X, and another cycle of the above calculation is carried out. This process is repeated until a fit is obtained which satisfies the program.

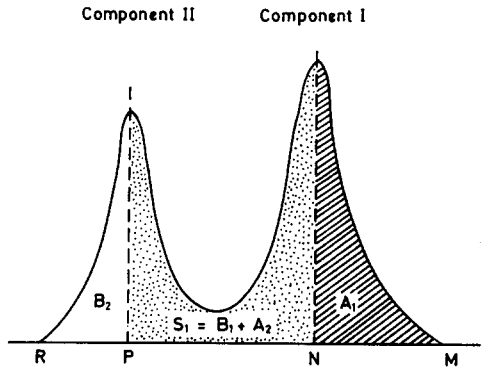

FIG. 3. The basis of estimating contributions of two components in a two-component peak complex. The area of component I is $A = A_1 + A_2$. The stippled area between the two maxima is $S_1 = B_1 + A_2$. The area of component II is $B_1 + B_2 = B$. For adjacent peaks it is assumed that $A_1/A = B_1/B$. This ratio is denoted by F in the text.

The majority of complexes has the same number of components as maxima, and the type of approach outlined above is used to estimate the contribution of each component. For single peaks with one extra component, it is assumed that the leading portion of a peak component contains 0.44 of its total area. This value has been used since under our conditions we have observed that single peaks are slightly skewed with, on the average, 44% of the area occurring between onset and maximum, 56% between maximum and tail. By subtraction from the total area, an estimate is obtained for the minor component. This situation is indicated in the print-out so that the user knows that this approximate method has been used.

For complexes with two or more maxima and an additional component on the trailing edge for example, the ratio of area before peak to total area of each major component except the last is estimated by summation from onset to first peak, from first peak to first trough, and so on. The mean estimated ratio is then applied to the area between the last trough and the last peak, and an approximation to the area of the minor component is obtained by difference. This situation is also indicated in the print-out.

The carbon numbers of the esters detected are identified from the linear relationship between carbon number and the logarithm of retention time established by Woodford and van Gent (3).

Under our conditions, methyl palmitate, when chromatographed on PEGA, always appears after 5.5 in. along the recorder chart, corresponding to the 330th point in the array; from our experience to date, this is

the first component after this point to give a peak height corresponding to at least 1 mv.

These criteria are used to detect methyl palmitate. The elution time of methyl stearate is 1.87–1.97 times that of palmitate. The identification of these two esters permits the program to estimate the equation which is then used to identify the carbon numbers of the other components present. A similar method is used for analyses on the Apiezon phase. The program is informed whether the sample has been analyzed on the PEGA or Apiezon phase by a character punched manually at the beginning of the sample tape.

The information printed for each component identified consists of the following: (*a*) the distance along the recorder chart where it appears (calculated from the relationship between sampling frequency of the data-logging system and the rate at which the chart is run), (*b*) the proportion of the component which appears before the maximum, (*c*) the area attributed to this component, (*d*) the percentage which it represents of the total esters detected, and (*e*) its carbon number. Occasionally the program fails to allocate carbon numbers if the amount of palmitate present is very small, but the information on distance along the recorder chart enables the user to identify the components. One further point should be noted: the identification of minor components is not an irrefutable assertion of their presence, but is rather an indication to the user that they might be present, and he may wish to investigate them further. Likewise, the estimates of area for these minor components are very approximate.

THE PROGRAM IN PRACTICE

The performance of the program is illustrated in this section of the paper. We were interested in comparisons between the results obtained from the computer using the program and those obtained by triangulation of the recorded peaks, in the repeatability of results, and in the range of GLC-loading over which the program could be relied upon to operate successfully.

Table 1 gives a summary of results of five analyses of a standard mixture of methyl esters on each of two phases. The values quoted are the means of the five analyses expressed for each methyl ester present as a percentage of the total esters in the sample. In parentheses beside the mean is the range of estimates obtained for each methyl ester. The two methods of estimation agree well, and the range is also similar, except possibly for 18:0 on the Apiezon phase where the computer estimates are more variable.

The results given in Table 2 are for another standard mixture which was chromatographed four times on the PEGA phase. In this case there was also good agreement.

TABLE 1 PERCENTAGE CONTRIBUTION, DETERMINED BY TRIANGULATION AND BY COMPUTER, TO TOTAL PEAK AREA OF INDIVIDUAL FATTY ACID METHYL ESTERS IN A MIXTURE OF SATURATED AND MONOUNSATURATED COMPONENTS

Fatty Acid	PEGA Liquid Phase		Apiezon Liquid Phase	
	Triangulation	Computer	Triangulation	Computer
14:0	11.0 (0.4)	11.2 (0.2)	10.7 (0.2)	11.3 (0.4)
16:0	25.1 (1.1)	24.7 (1.2)	25.0 (0.3)	24.8 (0.9)
16:1	6.7 (0.4)	7.1 (0.9)	6.3 (0.3)	6.3 (0.7)
18:0	12.4 (1.0)	12.2 (0.5)	12.8 (0.9)	13.0 (2.4)
18:1	44.5 (1.2)	44.5 (0.5)	45.3 (1.2)	44.6 (1.5)

The same sample was analyzed on both liquid phases. Values are means of five determinations, the range of which is given in parentheses.

TABLE 2 PERCENTAGE CONTRIBUTION, DETERMINED BY TRIANGULATION AND BY COMPUTER, TO TOTAL PEAK AREA OF INDIVIDUAL FATTY ACID METHYL ESTERS IN A MIXTURE OF SATURATED AND POLYUNSATURATED COMPONENTS

Fatty Acid	Triangulation	Computer
16:0	19.6 (0.6)	19.8 (0.7)
18:0	18.4 (0.7)	18.7 (0.8)
18:1	21.2 (0.3)	20.9 (0.7)
18:2	19.4 (0.2)	19.7 (0.4)
18:3	21.2 (0.7)	20.9 (1.0)

The sample was analyzed four times on PEGA, and values given are means with the range in parentheses.

TABLE 3 COMPUTER DETERMINATION OF COMPOSITION OF DIFFERENT AMOUNTS OF A MIXTURE OF FATTY ACID METHYL ESTERS

Fatty Acid	Amount on PEGA Column		Amount on Apiezon Column	
	12 μg	50 μg	30 μg	78 μg
14:0	10.9	10.9	11.4	11.3
16:0	25.5	25.8	25.3	24.2
16:1	6.9	6.6	6.1	6.4
18:0	12.6	11.9	12.7	13.8
18:1	44.1	44.8	44.4	44.1

Replicate analyses are obtained when amounts between 5 and 80 μg are used, provided that the detector responds linearly to each component.

The mixture used to illustrate the comparison of estimates by computer and triangulation was chromatographed at very different loads to examine the effect of load on estimates of concentrations of the different components.

The results are given in Table 3, and show typical effects of variation of load on the estimates obtained. Under our conditions the usual load is about 20 μg of methyl esters in ether, but we have obtained satisfactory reproducibility of results from 5 to 80 μg provided no one peak was outside the range of linearity of the detector.

Finally, we present in Table 4 typical computer output from the analysis of a data tape.

TABLE 4 SAMPLE PRINT-OUT FROM COMPUTER PROGRAM

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DATE OF RUN   6/ 6    RUN NO.   231

NO. OF VALUES  1601      APPARENT AREA   682.574

TYPE AND LOCATION OF PEAKS

NO. OF SINGLE PEAKS  10                                                    AREA

      1. 1        93       101       114                                  0.994
      1. 2       134       141       157                                  0.431
      1. 3       157       170       184                                  0.936
      1. 4       184       200       225                                 18.496
      1. 5       315       334       351                                  1.252
      1. 6       514       544       582                                 14.112
      1. 7       582       611       648                                  2.781
      1. 8       947      1042      1112                                 30.122
      1. 9      1121      1163      1171                                  1.875
      1.10      1257      1333      1343                                  3.607

NO. OF DOUBLE PEAKS   3

      2. 1       225       253       265       280       307             10.982
      2. 2       680       759       805       846       937            429.720
      2. 3      1379      1469      1480      1481      1516              9.163

NO. OF TRIPLE PEAKS   1

      3. 1       358       391       421       436       481       492       514   163.637

COMPLEX PEAKS

IN SINGLE PEAK   7  THERE ARE   3  COMPONENTS BETWEEN  10.0  AND  11.1  INCHES

IN SINGLE PEAK   8  THERE ARE   3  COMPONENTS BETWEEN  16.3  AND  19.1  INCHES

IN SINGLE PEAK   9  THERE ARE   3  COMPONENTS BETWEEN  19.2  AND  20.1  INCHES

IN SINGLE PEAK  10  THERE ARE   3  COMPONENTS BETWEEN  21.6  AND  23.1  INCHES

IN DOUBLE PEAK   3  THERE ARE   7  COMPONENTS BETWEEN  23.7  AND  26.0  INCHES

RESOLVED AREA AS PERCENTAGE OF APPARENT AREA   100.8

ORDERED COMPONENTS
ORDER   DIST.   AREA    RATIO   AREA PERCENT  REL.RET.TIME.  CARBON NO.  NUMBER

   1    1.73    0.99    0.46        0.1          0.26         11.92       1. 1
   2    2.42    0.43    0.45        0.1          0.36         12.92       1. 2
   3    2.92    0.94    0.44        0.1          0.43         13.49       1. 3
   4    3.43   18.50    0.47        2.7          0.51         13.98       1. 4
   5    4.09    1.32   -0.99        0.2          0.61         14.50       2. 1
   6    4.34    2.91    0.49        0.4          0.65         14.69       2. 1
   7    4.81    6.75    0.49        1.0          0.72         14.99       2. 1
   8    5.73    1.25    0.55        0.2          0.85         15.52       1. 5
   9    6.71  138.45    0.49       20.1          1.00         16.00       3. 1
  10    7.49   21.70    0.49        3.2          1.12         16.33       3. 1
  11    8.45    3.64    0.49        0.5          1.26         16.69       3. 1
  12    9.34   14.11    0.47        2.1          1.39         17.00       1. 6
  13   10.49    2.78    0.44        0.4          1.56         17.35       1. 7
  14   11.77    3.25   -0.99        0.5          1.75         17.69       2. 2
  15   13.03  235.30    0.46       34.2          1.94         18.00       2. 2
  16   14.53  191.17    0.46       27.8          2.16         18.33       2. 2
  17   17.89   30.12    0.53        4.4          2.66         18.96       1. 8
  18   19.97    1.88    0.81        0.3          2.97         19.29       1. 9
  19   22.89    3.61    0.76        0.5          3.41         19.70      1.10
  20   25.22    6.80    0.82        1.0          3.76         19.99       2. 3
  21   25.43    2.37    0.04        0.3          3.79         20.02       2. 3
```

The Apparent Area is the area above the linear base line. Under Type and Location of Peaks, the peak number is followed by the position in the array of onset, maxima, troughs and tail, and the area corrected if necessary for negative base line. Under Ordered Components the ratio represents the proportion of the peak area occurring before the maximum. Entries of −0.99 for the ratio indicate components estimated by difference (see text).

The material chromatographed by PEGA was methyl esters of fatty acids from perinephric adipose tissue of a moose calf. This table illustrates most aspects of the program. The print-out identifies the sample and estimates the apparent area from a summation of points above the linear base line. We have retained the section of output giving the types and locations of peaks. These are typed according to the number of maxima detected in each complex. Single peak 1.1 has its onset at the 93rd point in the array, the maximum occurs at point 101, and the complex returns to base line at point 114. The area is 0.994; it is sometimes of interest to the user to

know the length of a peak and whether there is a long tail.

The next information given concerns those complexes for which the program is unable to estimate the contribution of individual components. This information comes under the heading Complex Peaks. In single peak 7, for example, the program asserts that there are three components between 10.0 and 11.1 in. from the onset of the solvent peak on the recorder chart. This statement is made with the reservations outlined in the previous section. Under the heading Ordered Components, the program attributes the entire area of this peak (1.7) to the component associated with the maximum at point 611; this area is 2.78, and the proportion of this complex before the maximum is 0.44, which might lead one to believe that there was in fact only one component.

In the first double peak (2.1), three components were detected, the minor one occurring on the leading edge. The area of the third component (6.75) was estimated by summations from tail to last maximum and from last maximum to trough. These summations were used to estimate the proportion (0.49) of this component occurring before the maximum. The proportion 0.49 was applied to the area from the trough to the first maximum to estimate the area (2.91) of the first major component. The area of the leading minor component was estimated by subtracting 6.75 and 2.91 from 10.98 to obtain 1.32. The fact that it is the minor component and is estimated by this crude method is indicated by the entry −0.99 under Ratio in the print-out. Component 14, a leading minor component in the second double peak, was similarly estimated.

On the other hand components 9, 10, and 11 were the only components detected in the triple-peak complex, and their areas were estimated by the iterative procedure illustrated in the previous section of this paper.

In contrast with component 13 (single peak 1.7), component 18 (single peak 1.9) is alleged to have three components. The fact that the ratio of 0.81 is so different from the usual value of approximately 0.44, suggests that there might well be more than one component in this peak, although the area is very small at 1.88, and there is a tendency for less regularly shaped peaks towards the end of the run.

Carbon numbers are determined by relative retention time, and zero time is at the onset of the solvent peak. As the program stands at present, the onset is taken as the first point in time when the reading on the digital voltmeter exceeds 7.0 mv. There may be slight variation from one sample to another in the rate of rise of the solvent peak with a consequent slight error in retention times and carbon numbers, more noticeable among the earlier components, but we have never found this to be greater than about 0.02.

Finally, the "resolved area as a percentage of the apparent area" may differ slightly from 100 in either direction. It may be greater if some of the resolved peaks start from negative values, and smaller if there are peaks of less than 16 sec in duration.

COMPARISON WITH OTHER METHODS

Other papers have been written on the interpretation of GLC output, but most of these describe methods for problems of quite a different nature. Buchanan and Maher (4), for example, describe a program to facilitate the analysis of complex mixtures by gas chromatography where the sample has been analyzed before and after treatments designed to remove successively olefins, aromatics, and normal paraffins. Many papers describe the resolution of qualitative rather than quantitative analyses; the paper by Biggers, Hilton, and Gianturco (5) is typical of these.

In the approach adopted by Bentsen and Bethea (6) for short-chain fatty acids, several columns are necessary in addition to a considerable amount of basic data for all the compounds contained in an information library with which the unknowns are compared. It is also necessary to punch on data-cards a description of each peak obtained on each chromatogram.

Caster, Ahn, and Pogue (7) describe a computer method for GLC quantitation of fatty acid esters, and this work is most nearly comparable in its aims to the work reported in this paper. Their program requires operator intervention to the extent of physical mensuration of peaks on the recorder chart and of punching cards, one for each component, giving its peak height and retention time. It also requires constants obtained from a series of standard mixtures of known composition.

In contrast with these methods, the program reported here does not require operator intervention, which involves delay and the possibility of human error, as the output from the chromatograph, automatically punched on paper tape, is fed directly into the computer. No external standards are necessary as we are able to rely on the identification of methyl palmitate and methyl stearate to determine, for each component, its relative retention time and carbon number. Under other conditions any two appropriate esters would suffice, provided they were reasonably separated on the column.

While many components of a mixture of fatty acid esters can be presumptively identified from a single chromatogram, it is often necessary as a further check on identity to compare the behavior of a component with respect to relative retention time on two different liquid phases, and when unsaturated components are present, to chromatograph a fully hydrogenated sample.

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