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# A PRACTICAL QUANTITATIVE METHOD FOR UNRESOLVED GAS CHROMATOGRAPHIC PEAKS

## I. TWO OVERLAPPING PEAKS

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SUMMARY

A manual quantitative analysis of overlapping peaks, of Gaussian or non-Gaussian shapes and at various resolutions, was carried out by the peak height ratio method, in which the true peak height is obtained by subtracting the contribution of the overlapping peak. Each peak could be evaluated as accurately as a resolved peak, but when overlapping was severe an error of several per cent occurred in the analytical value for the tailing peak, although only when the amount of component in this peak was comparable with that in the leading peak.

## INTRODUCTION

Quantitative analysis by gas chromatography (GC) is simple to carry out when peaks are completely separated. For unresolved peaks, the perpendicular or skimming methods are well known, but values obtained by these methods are not very precise[1,2]. Recently, computer-assisted analysis has been applied to a variety of problems in chromatographic analysis[1-8]. This method utilizes a curve-fitting technique, in which the most suitable function for the peak shape under study must first be determined. A GC peak with no tailing is assumed to be Gaussian in shape. However, peaks often show various degrees of tailing, especially on a low-loaded packed column. When the area of a peak is calculated from the product of its height and its $\sigma$ value in the curve-fitting technique, the value obtained for a minor peak on the tailing edge can have a large error. For computer analysis, the detection of overlapping peaks is a primary requirement, but when the peak for a minor component slightly overlaps a large peak or when moderate components are severely overlapped, the curve is often smooth and uninflected, having no separate peaks or even a shoulder, thus rendering peak detection of the minor component impossible. This paper describes a simple calculation method for unresolved GC peaks, including both Gaussian and non-Gaussian peaks of different resolutions.

## THEORETICAL

An unresolved chromatogram of a mixture of compounds 1 and 2 is illustrated schematically in Fig. 1. If the peak heights of the resolved components 1 and 2

are $H_1$ and $H_2$, respectively, the difference between retention times $T_1$ and $T_2$ on the chromatogram is $l$, the contributions of each peak to the height of the other peak are $H_{12}$ and $H_{21}$, respectively, and the peak heights at $T_1$ and $T_2$ are $h_1$ and $h_2$,

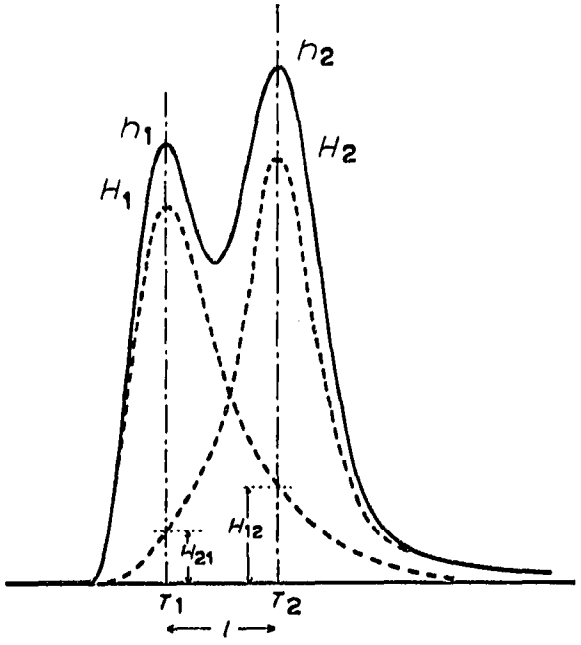


Fig. 1. Unresolved chromatogram of a mixture of compounds 1 and 2 (schematic). For explanation, see text.

respectively, then, assuming that there is no displacement in retention time between the maximum of the larger peak and that of the corresponding single peak, the height of the minor component is obtained from the height at the point $l$ units away from the maximum of the larger peak, and the following pair of equations is obtained:

$$h_1 = H_1 + H_{21}$$
$$h_2 = H_2 + H_{12} \qquad (1)$$

$H_{mn}$ are calculated from the chromatograms of the pure components $m$ and $n$. If the height of the resolved peak of the leading component of the fused peak is $H_1$, the height at the point $l$ units away from the peak maximum towards the tailing edge is $H_{12}$, and for the peak on the tailing edge in a fused peak the height of the contribution is $H_{21}$ at the site of the leading edge, then:

$$a_m = \frac{H_{mn}}{H_m} \qquad (2)$$

where $a_m$ is referred to as the overlapping coefficient. Using this, eqns. 1 can be written as:

$$h_1 = H_1 + a_2 H_2$$
$$h_2 = H_2 + a_1 H_1 \qquad (3)$$

Accordingly:

$$H_1 = \frac{h_1 - a_2 h_2}{1 - a_1 a_2}$$
$$H_2 = \frac{h_2 - a_1 h_1}{1 - a_1 a_2} \quad (4)$$

If $a_2 = 0$, eqns. 4 reduce to:

$$H_1 = h_1$$
$$H_2 = h_2 - a_1 h_1 \quad (5)$$

If the product of $a_1$ and $a_2$ is negligibly small, eqns. (4) simplify to

$$H_1 = h_1 - a_2 h_2$$
$$H_2 = h_2 - a_1 h_1 \quad (6)$$

## EXPERIMENTAL

The analyses were carried out with the flame ionization detector of a Shimadzu GC-4APF gas chromatograph. The glass columns used were 4 mm in diameter and were packed with Gas-Chrom Q (100–120 mesh) coated with OV-1. The other parameters were as given in Table I.

TABLE I

GAS CHROMATOGRAPHIC OPERATING CONDITIONS

| *System* | *Column* | | *Nitrogen carrier-gas pressure* ($kg/cm^2$) | *Temperature* (°C) | | |
|---|---|---|---|---|---|---|
| | *Phase* (%) | *Length* (*m*) | | *Column* | *Injector* | *Detector* |
| *n-Eicosane–androstane* | | | | | | |
| Overlapping | 3 | 3 | 1.8 | | 240 | 260 |
| Perfect resolution | 3 | 3 | 1.1 | 242 | 242 | 260 |
| *5α-Androst-3, 11, 17-trione–n-hexacosane* | | | | | | |
| Overlapping | 1 | 2 | 2.0 | | 240 | 310 |
| Perfect resolution | 1 | 2 | 0.8 | 279.6 | 290 | 310 |

## RESULTS AND DISCUSSION

### *Gaussian–Gaussian peaks*

An *n*-eicosane–androstane system in which the former component elutes first on an OV-1 column at the temperature studied was well separated at a column temperature of 242°, and the degree of resolution decreased with decreasing column temperature. All analyses were carried out on the same column. We chose three

column temperatures at which the curve parameters fitted eqns. 4, 5 and 6, respectively. It can be seen in Table II, that at a column temperature of 207.5° the overlapping coefficient for androstane was negligibly small, allowing eqns. 5 to be used; at 204.6°, the product of the overlapping coefficients was negligibly small, allowing eqns. 6 to be used; under the most severely overlapping condition, at a column temperature of 202.6°, resolution using half-height width was less than unity, requiring the use of eqns. 4. *n*-Octadecane was used as an internal standard, and the amount of androstane relative to *n*-eicosane in the system was varied over the range from 5 % to over 100 %.

TABLE II

RELATIONSHIPS BETWEEN COLUMN TEMPERATURE AND RESOLUTION AND OVERLAPPING COEFFICIENT

| *Column temperature* (°C) | *Retention time* $R_T$ (*min*) | | | *Resolution*[a] | *Overlapping coefficient* | | *Product of overlapping coefficients* |
|---|---|---|---|---|---|---|---|
| | $C_{18}$ | $C_{20}$ | *Androstane* | | $C_{20}$ | *Androstane* | |
| 207.5 | 2.59 | 5.23 | 5.58 | 1.58 | 0.0160 | 0 | 0 |
| 204.6 | 3.03 | 6.19 | 6.49 | 1.23 | 0.0400 | 0.0164 | 0.0007 |
| 202.6 | 3.60 | 7.41 | 7.69 | 0.97 | 0.0927 | 0.0776 | 0.0719 |

[a] Calculated from width at half peak-height.

Chromatograms obtained for the analysis of *n*-eicosane at the three resolutions are shown in Fig. 2. When the resolution was 1.58, well defined maxima occurred on each chromatogram. When the resolution was 1.23, mixtures 1–4 showed two maxima, but the mixtures having an *n*-eicosane content lower than that of mixture 4 exhibited a shoulder peak. At a resolution of 0.97, all mixtures showed shoulders or uninflected peaks, except for mixture 1. It is surprising that, even for heavily overlapping peaks, the degrees of overlapping are less than 10 % of the peak height. This result indicates the validity of our method. Analytical data obtained from Fig. 2 are summarized in Table III. As our quantitative method is based on the peak-height

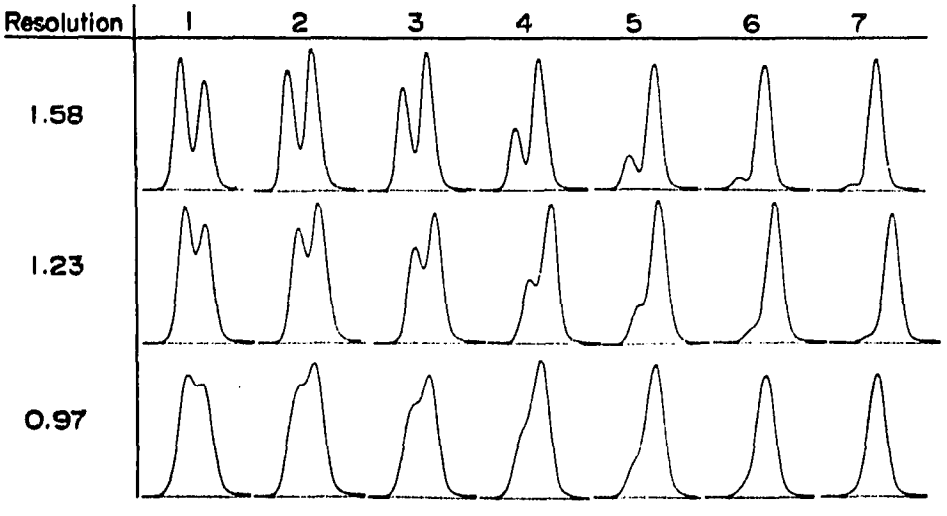


Fig. 2. Overlapping chromatograms for mixtures of *n*-eicosane and androstane at three resolutions.

ratio method, the retention time relative to the internal standard may vary with the column temperature. Therefore, any given sample may give different results at different column temperatures. This is shown in the values obtained at different resolutions in Table III. Quantitative values had a very low standard deviation (<0.4 %), even under the heavily overlapping conditions (0.9 % at $R = 0.97$). Response factors, calculated from the ratio of the observed value at any given resolution to the value at perfect resolution, had a standard deviation of only 0.6 % at any resolution, except when the concentration of *n*-eicosane was low. As seen from Table III and Fig. 3, the quantitative analysis of *n*-eicosane, the leading peak of the overlapping pair, could be made even at a resolution of unity.

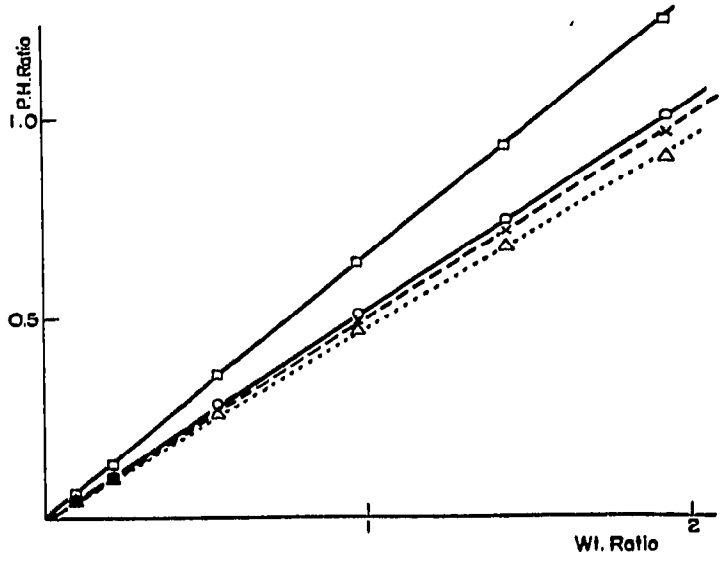


Fig. 3. Regression lines for *n*-eicosane. Internal standard: *n*-octacosane. Column temperature (°C): ○, 207.5; ×, 204.6; △, 202.6; □, 242. Resolution: ○, 1.58; ×, 1.23; △, 0.97.

For the quantitative analysis of androstane, the tailing peak of the overlapping pair, three resolutions were selected for use in eqns. 4, 5 and 6, as for *n*-eicosane. However, the peak maximum of androstane, as seen in Table II, did overlap with the tail of the *n*-eicosane peak, even at a resolution of 1.58, although the chromatograms were almost identical to those for *n*-eicosane except for the greater degree of overlapping. The analytical values for androstane are shown in Table IV and Fig. 4. At resolutions of 1.58 and 1.23, the standard deviation of each mixture was less than 0.7 %, and the response factors relative to the values for the resolved peaks showed only small deviations. When the resolution was decreased to 0.97, however, lower values were obtained on increasing the proportion of androstane in the system to over 50 %, although at 109 % of androstane the values were higher than the theoretical values. These results are a consequence of a shift in the position of the peak maximum. When androstane is the minor component, the observed peak maximum for *n*-eicosane shifts backwards. Therefore, the apparent retention time of androstane measured from *n*-eicosane is longer than the true value, resulting in a lower quantitative value. However, when androstane is the major component, where the maximum of the *n*-eicosane peak is determined from the maximum of the androstane

TABLE III

ANALYTICAL VALUES FOR *n*-EICOSANE AT DIFFERENT RESOLUTIONS

| System | Weight ratio (%) | | Resolution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $C_{20}/C_{18}$ | $C_{20}$/Androstane | 1.58 | | | 1.23 | | | 0.97 | | |
| | | | Peak height ratio $C_{20}/C_{18}$ (%) | Standard deviation (%, $n = 5$) | Response factor[a] | Peak height ratio $C_{20}/C_{18}$ (%) | Standard deviation (%, $n = 5$) | Response factor[a] | Peak height ratio $C_{20}/C_{18}$ (%) | Standard deviation (%, $n = 5$) | Response factor[a] |
| 1 | 190.5 | 128.0 | 100.8 | 0.32 | 80.0 | 97.9 | 0.20 | 77.8 | 89.1 | 0.59 | 71.0 |
| 2 | 193.2 | 90.53 | 100.8 | 0.28 | 80.5 | 96.9 | 0.16 | 77.4 | 90.6 | 0.89 | 72.4 |
| 3 | 143.7 | 93.85 | 74.8 | 0.12 | 79.7 | 72.4 | 0.36 | 77.1 | 67.9 | 0.36 | 72.3 |
| 4 | 97.84 | 48.72 | 51.1 | 0.10 | 79.8 | 49.1 | 0.10 | 76.7 | 46.8 | 0.35 | 73.1 |
| 5 | 54.09 | 27.82 | 28.3 | 0.10 | 79.3 | 27.2 | 0.19 | 76.2 | 25.8 | 0.61 | 72.3 |
| 6 | 19.92 | 10.01 | 10.2 | 0.12 | 79.1 | 9.9 | 0.21 | 76.7 | 9.3 | 0.50 | 72.1 |
| 7 | 9.06 | 5.17 | 4.6 | 0.05 | 80.7 | 4.5 | 0.21 | 78.9 | 3.8 | 0.25 | 66.7 |

[a] Peak-height ratio at the given resolution over ratio at perfect resolution.

TABLE IV

ANALYTICAL VALUES FOR ANDROSTANE AT DIFFERENT RESOLUTIONS

| System | Weight ratio (%) | | Resolution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Androstane/$C_{18}$ | Androstane/$C_{20}$ | 1.58 | | | 1.23 | | | 0.97 | | |
| | | | Peak height ratio, Androstane/$C_{18}$ (%) | Standard deviation (%, $n = 5$) | Response factor | Peak height ratio, Androstane/$C_{18}$ (%) | Standard deviation (%, $n = 5$) | Response factor[a] | Peak height ratio, Androstane/$C_{18}$ (%) | Standard deviation (%, $n = 5$) | Response factor[a] |
| 2 | 211.4 | 109.4 | 116.6 | 0.73 | 95.5 | 115.9 | 0.29 | 94.9 | 119.8 | 0.26 | 98.1 |
| 1 | 148.9 | 78.15 | 82.0 | 0.21 | 95.1 | 82.1 | 0.47 | 95.2 | 77.4 | 0.65 | 89.8 |
| 11 | 108.5 | 51.90 | 60.4 | 0.32 | 95.4 | 60.1 | 0.22 | 94.9 | 58.4 | 0.53 | 92.3 |
| 12 | 52.08 | 26.42 | 29.0 | 0.29 | 95.7 | 29.1 | 0.65 | 96.0 | 28.2 | 0.30 | 93.1 |
| 13 | 30.69 | 14.12 | 17.3 | 0.05 | 97.7 | 17.1 | 0.21 | 96.6 | 16.9 | 0.41 | 95.5 |
| 14 | 12.99 | 6.43 | 7.2 | 0.10 | 94.7 | 7.3 | 0.16 | 96.1 | 7.2 | 0.22 | 94.7 |

peak, the peak height of *n*-eicosane is under-evaluated, resulting in a higher quantitative value for androstane.

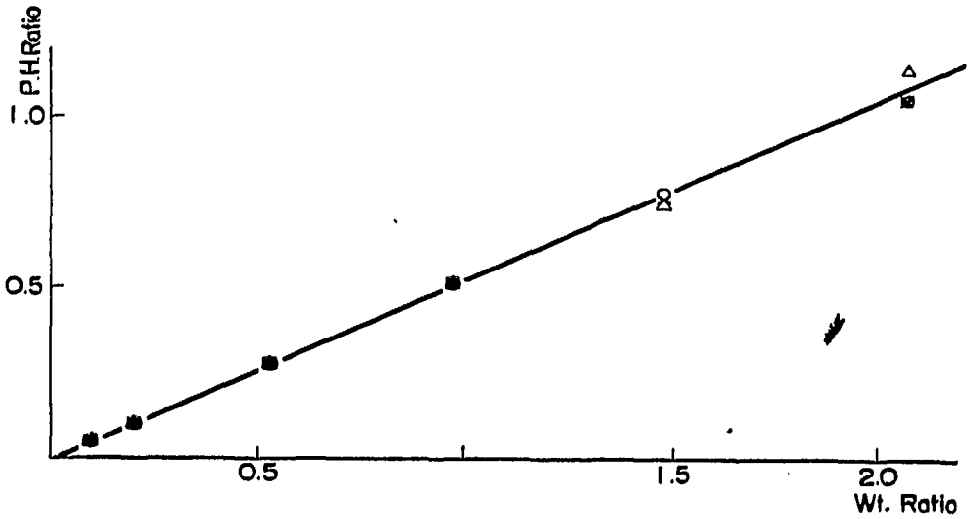


Fig. 4. Regression line for androstane. Internal standard: *n*-octacosane. Column temperature (°C): ○, 207.5; ×, 204.6; △, 202.6; □, 242. Resolution: ○, 1.58; ×, 1.23; △, 0.97.

*Non-Gaussian–Gaussian peaks*

A mixture of 5α-androst-3,11,17-trione (KA) and *n*-hexacosane, in which the former elutes with tailing, was well separated at a column temperature of 279.6°. In this case, the first eluate was *n*-hexacosane, but the order of elution was reversed under the overlapping conditions studied. Three resolutions were selected as shown in Table V. The overlapping coefficients of KA were very high because of tailing, The analyses were carried out using *n*-tetracosane as an internal standard. Analytical data for KA are shown in Table VI and plotted in Fig. 5. The amount of KA in the system relative to *n*-hexacosane was varied over the range 10–149%. Although the overlapping coefficients for KA were high, the analytical values obtained were as good as those for *n*-eicosane in the two Gaussian types mentioned above, with regard to accuracy, precision and the response factors relative to the values at perfect resolution. The chromatograms obtained for the analysis of *n*-hexacosane, which is the tailing peak of the fused pair, are illustrated in Fig. 6. Although mixtures 11–13 at a resolution of 1.31 exhibited well defined peak maxima, mixtures 11 and 12 at a resolution of 1.23 showed only a shoulder. At a resolution of 0.92, mixture 15

TABLE V

RELATIONSHIPS BETWEEN COLUMN TEMPERATURE AND RESOLUTION AND OVERLAPPING COEFFICIENT

| *Column temperature* (°C) | *Retention time, $R_T$ (min)* | | | *Resolution*[a] | *Overlapping coefficient* | |
|---|---|---|---|---|---|---|
| | *n-C24* | *KA* | *n-C26* | | *KA* | *n-C26* |
| 233.5 | 4.29 | 7.46 | 7.91 | 1.27 | 0.1806 | 0.0096 |
| 234.6 | 4.02 | 7.01 | 7.38 | 1.12 | 0.2080 | 0.0289 |
| 236.4 | 3.90 | 6.77 | 7.05 | 0.92 | 0.2485 | 0.1001 |

[a] Calculated with width at half peak-height.

TABLE VI

ANALYTICAL VALUES FOR 5α-ANDROST-3, 11, 17-TRIONE (KA) AT DIFFERENT RESOLUTIONS

| System | Weight ratio (%) | | Resolution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | KA/C24 | KA/C26 | 1.27 | | | 1.12 | | | 0.92 | | |
| | | | Quantitative value, KA/C24 (%) | Standard deviation (%, n = 5) | Response factor[a] to resolved peak | Quantitative value, KA/C24 (%) | Standard deviation (%, n = 5) | Response factor to resolved peak | Quantitative value, KA/C24 (%) | Standard deviation (%, n = 5) | Response factor[a] to resolved peak |
| 1 | 307.9 | 148.9 | 107.1 | 0.35 | 1.21 | 105.6 | 0.63 | 1.19 | 106.3 | 1.23 | 1.20 |
| 2 | 204.1 | 100.8 | 69.6 | 0.59 | 1.23 | 69.1 | 0.58 | 1.22 | 71.5 | 1.13 | 1.27 |
| 3 | 134.6 | 75.94 | 47.9 | 0.46 | 1.25 | 48.0 | 0.67 | 1.26 | 50.3 | 0.83 | 1.32 |
| 4 | 98.65 | 51.24 | 30.2 | 0.47 | 1.27 | 30.0 | 0.13 | 1.26 | 32.8 | 0.89 | 1.38 |
| 5 | 49.30 | 25.40 | 12.7 | 0.29 | 1.27 | 12.0 | 0.31 | 1.20 | 12.2 | 0.57 | 1.22 |
| 6 | 21.05 | 10.81 | 3.0 | 0.40 | | 3.5 | 0.47 | | 3.6 | 0.71 | |

[a] Peak-height ratio at the given resolution over ratio at perfect resolution.

TABLE VII

ANALYTICAL VALUES FOR *n*-HEXACOSANE AT DIFFERENT RESOLUTIONS

| System | Weight ratio (%) | | Resolution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | C26/C24 | C26/KA | 1.31 | | | 1.16 | | | 0.95 | | |
| | | | Quantitative value, C26/C24 (%) | Standard deviation (%, n = 5) | Response factor[a] to resolved peak | Quantitative value, C26/C24 (%) | Standard deviation (%, n = 5) | Response factor[a] to resolved peak | Quantitative value, C26/C24 (%) | Standard deviation (%, n = 5) | Response factor to resolved peak |
| 11 | 206.8 | 67.17 | 106.7 | 0.45 | 0.835 | 106.7 | 0.60 | 0.835 | 114.8 | 0.54 | 0.892 |
| 12 | 149.6 | 49.85 | 77.8 | 0.52 | 0.815 | 78.1 | 0.63 | 0.819 | 74.8 | 1.25 | 0.785 |
| 13 | 97.75 | 32.19 | 51.5 | 0.36 | 0.815 | 51.9 | 0.60 | 0.822 | 51.1 | 0.46 | 0.808 |
| 14 | 53.10 | 19.42 | 27.7 | 0.20 | 0.810 | 28.1 | 0.93 | 0.822 | 27.7 | 0.23 | 0.811 |
| 15 | 20.09 | 6.42 | 10.0 | 0.10 | 0.795 | 10.6 | 0.52 | 0.841 | 10.7 | 0.26 | 0.848 |
| 16 | 10.03 | 3.39 | 4.6 | 0.24 | | 5.0 | 0.23 | | 5.7 | 0.53 | |

exhibited a smooth, non-inflected curve. These features were almost the same as those in Fig. 2 for the gas chromatograms of the *n*-eicosane–androstane system.

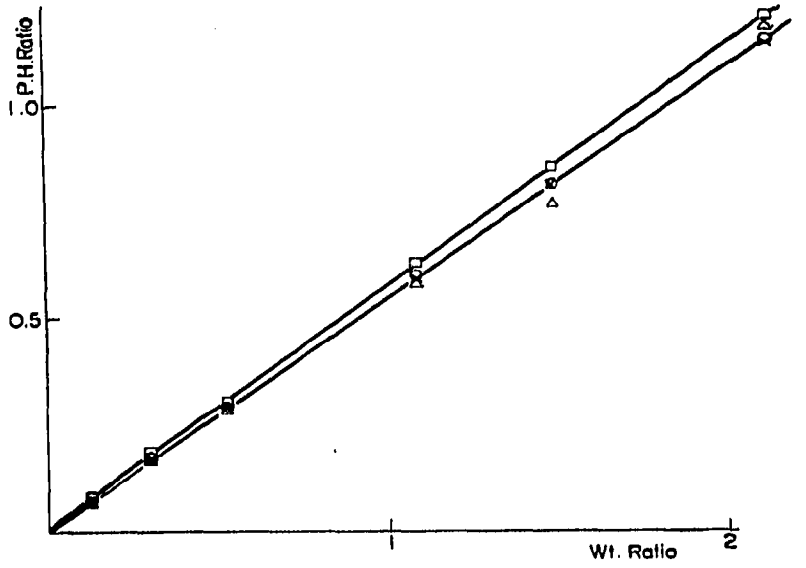


Fig. 5. Regression lines for 5α-androstan-3,11,17-trione. Internal standard: *n*-tetracosane. Column temperature (°C): ○, 233.5; △, 234.6; ×, 236.4; □, 279.6. Resolution: ○, 1.27; △, 1.12; ×, 0.92.

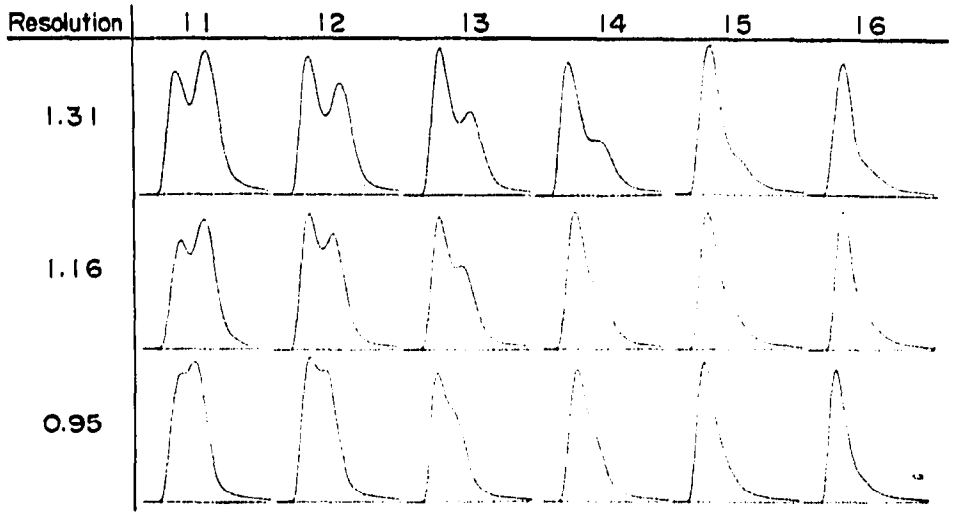


Fig. 6. Overlapping chromatograms for mixtures of 5α-androst-3,11,17-trione and *n*-hexacosane at three resolutions.

The analytical data for *n*-hexacosane eluted on the tailing edge peak are summarized in Table VII and its regression lines are shown in Fig. 7. Accuracies and standard deviations were as good as those obtained for androstane in the Gaussian–Gaussian-type system. At a resolution of 0.95, the quantitative amount of *n*-hexacosane in mixture 12 deviated to less than the theoretical value, but mixture 11 showed a positive deviation as seen in the androstane system, as a result of displacement of the peak maximum. Precise values for *n*-hexacosane were obtained, even when overlapping was severe.

The quantitative values for a non-Gaussian peak in a Gaussian–non-Gaussian system, in the elution order, are obtained by the same treatment as for a Gaussian–Gaussian system. A non-Gaussian–non-Gaussian system can also be evaluated as for a non-Gaussian–Gaussian system, as our method is not affected by the shape of the peak at the tailing edge. Our simple calculation method has therefore been proved to be suitable for the quantitative analysis of the components of overlapping peaks,

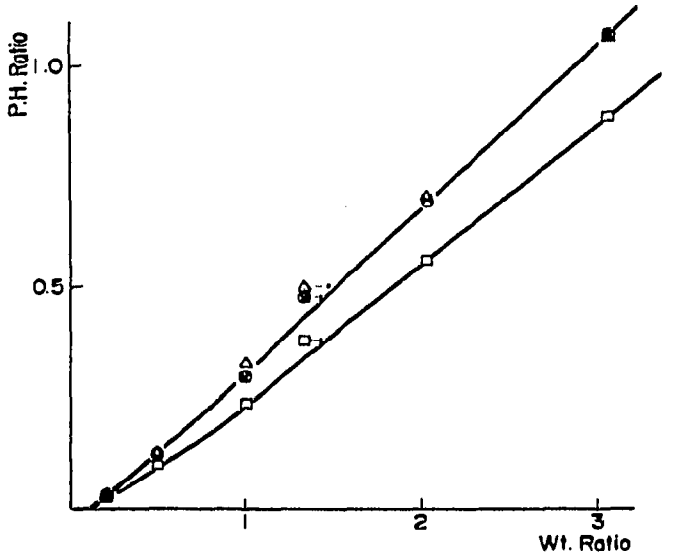


Fig. 7. Regression lines for *n*-hexacosane. Internal standard: *n*-tetracosane. Column temperature (°C): ○, 233.5; ×, 234.6; △, 236.4. Resolution: ○, 1.27; ×, 1.12; △, 0.92.

either Gaussian or non-Gaussian, giving an accuracy as high as that for resolved peaks. A deviation was found, however, when both components had comparable peak heights and a resolution of unity. These defects could be eliminated by the addition of a given weight of either component or addition of a marker that has a retention time near to that of the overlapping peaks. The choice of the appropriate conditions for perfect resolution from the wide range of techniques available is often difficult and time-consuming: this simple quantitative method will make analysis by GC more widely applicable.

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