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A PRACTICAL QUANTITATIVE METHOD FOR UNRESOLVED GAS CHROMATOGRAPHIC PEAKS

II. THREE OVERLAPPING PEAKS

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SUMMARY

Graphical analyses of three overlapping peaks at three resolutions were carried out by the peak height ratio method in which the true peak height is obtained by subtracting the contributions of the other overlapping peaks. Even at unit resolution, good accuracies were obtained and standard deviations were less than one unit per cent.

INTRODUCTION

Quantitative analysis of overlapping peaks has been developed using a computer to simulate the experimental data with Gaussian[1-7] or non-Gaussian[3,5-8] peak shapes. We have proposed simple equations for the quantitative analysis of two overlapping peaks[9], the application of which does not require definition of the peak shape, and results in an accuracy as high as that obtained for peaks with perfect resolution. By this method, the results are obtained graphically and the equations can be solved by manual calculation. This paper describes the application of this manual method to three overlapping peaks, consisting of two non-Gaussian peaks and a Gaussian peak, at various resolutions. Fig. 1 illustrates the three overlapping peaks schematically. The elution order is 1, 2, 3. Given that the retention times are T_1, T_2 and T_3, the peak heights of the components are H_1, H_2 and H_3, the observed heights at the positions of T_1, T_2 and T_3 are h_1, h_2 and h_3, respectively, and the overlapping coefficient[9] of peak m to peak n is a_{mn}, then

$$H_1 = h_1 - H_{21} \tag{1}$$

$$H_2 = h_2 - H_{12} - H_{32} \tag{2}$$

$$H_3 = h_3 - H_{13} - H_{23} \tag{3}$$

where

$$H_{12} = a_{12}H_1, \quad H_{13} = a_{13}H_1, \quad H_{21} = a_{21}H_2, \quad H_{23} = a_{23}H_2, \quad H_{32} = a_{32}H_3$$

Accordingly, we obtain

$$H_1 = h_1 - a_{21} \cdot \frac{(h_2 - a_{12}h_1) - a_{32}(h_3 - a_{13}h_1)}{1 - a_{12}a_{21} - a_{23}a_{32} + a_{13}a_{21}a_{32}} \tag{4}$$

$$H_2 = \frac{(h_2 - a_{12}h_1) - a_{32}(h_3 - a_{13}h_1)}{1 - a_{12}a_{21} - a_{23}a_{32} + a_{13}a_{21}a_{32}} \quad (5)$$

$$H_3 = h_3 - a_{13}h_1 - (a_{23} - a_{13}a_{21}) \cdot \frac{(h_2 - a_{12}h_1) - a_{32}(h_3 - a_{13}h_1)}{1 - a_{12}a_{21} - a_{23}a_{32} + a_{13}a_{21}a_{32}} \quad (6)$$

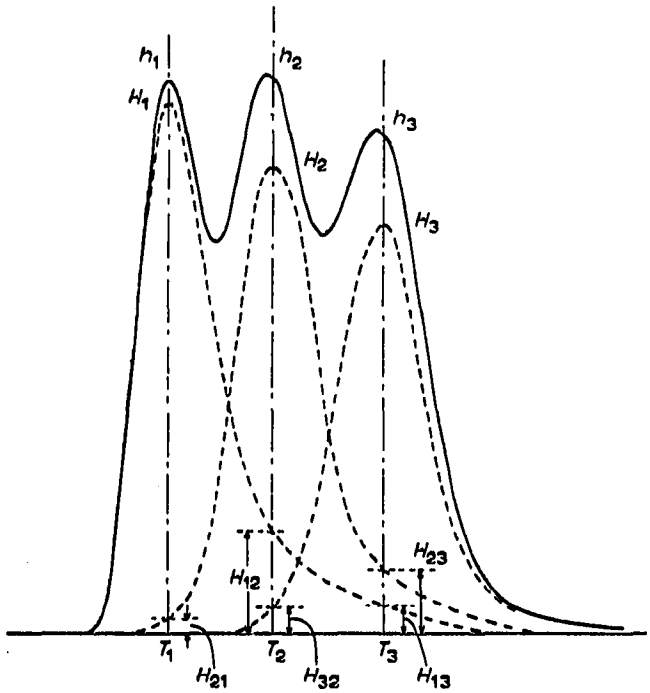

Fig. 1. Schematic illustration of two non-Gaussian and one Gaussian peak.

EXPERIMENTAL

The analyses were carried out with a flame ionization detector and a Shimadzu GC-4APF gas chromatograph. The glass column used was 1.3 m long and of 4 mm I.D. and was packed with Gas-Chrom Q (100–120 mesh) coated with 1 % OV-1. The injection and detector temperatures were 305° and 310°, respectively. The carrier gas was nitrogen at a pressure of 1.8 kg/cm^2. The mixtures in Table I were dissolved in dichloroethane (2 ml) and 1-μl volumes were injected with a 10-μl Hamilton syringe. Overlapping coefficients and quantitative values were obtained as a mean of four determinations in which the chromatograms were run at a chart speed of 4 cm/min.

RESULTS AND DISCUSSION

The mixture we used was composed of 5α-androst-3,11,17-trione (K_3A), 3α,17β-dihydroxy-5α-androst-11-one (H_2KA) and *n*-hexacosane, in that elution order; *n*-tetracosane was used as an internal standard. The ratio of the amounts of *n*-tetracosane, K_3A and *n*-hexacosane was constant in all the mixtures, while the amount of H_2KA was varied over the range 25.7–381 % with respect to *n*-tetracosane (Table I). Gas chromatograms of K_3A and H_2KA showed considerable tailing. Resolutions were dependent on column temperature. Table II summarizes the chromatographic

TABLE I

WEIGHT COMPOSITIONS OF MIXTURES

Substance	*Mixture*					
	1	*2*	*3*	*4*	*5*	*6*
C_{24} (I) (mg)	1.182	1.182	1.182	1.182	1.182	1.182
K_3A (II) (mg)	3.012	3.012	3.012	3.012	3.012	3.012
C_{26} (III) (mg)	2.266	2.266	2.266	2.266	2.266	2.266
H_2KA (IV) (mg)	4.505	3.004	2.253	1.502	0.751	0.303
IV/I (%)	381.1	254.1	190.5	127.0	63.5	25.7
II/IV (%)	66.9	100.3	133.7	200.6	401.2	992.8
III/IV (%)	50.3	75.5	100.6	150.9	301.8	746.9

TABLE II

RELATIONSHIP OF COLUMN TEMPERATURE WITH RESOLUTION AND OVERLAPPING COEFFICIENT

Parameter	*Column temperature (°C)*		
	213.7	*215.5*	*217.3*
R_T (min)			
n-C_{24}	4.08	3.86	3.71
K_3A	6.48	6.24	5.99
H_2KA	7.16	6.84	6.59
n-C_{26}	7.90	7.48	7.11
Resolution[a]			
(K_3A–H_2KA)	1.25	1.20	1.06
(H_2KA–n-C_{26})	1.18	1.08	0.932
Overlapping coefficient			
a_{12}	0.1078	0.1164	0.1126
a_{13}	0.0153	0.0187	0.0201
a_{21}	0	0	0
a_{23}	0.0858	0.1078	0.1555
a_{32}	0.0203	0.0439	0.0952

[a] Calculated from width at half peak height.

parameters at three column temperatures, the retention time, the resolution calculated from the peak width at half peak height, and the overlapping coefficient. Resolutions of K_3A–H_2KA and H_2KA–n-hexacosane decreased from 1.25 to approximately unity with increasing column temperature. The overlapping coefficient a_{21} of H_2KA with K_3A was zero. The overlapping coefficient a_{12} of K_3A was high, but changed slightly with increasing resolution. On the contrary, a_{23} and a_{32} increased markedly with decreasing resolution. Chromatograms of each mixture at the three resolutions are illustrated in Fig. 2. At the highest resolution studied, the positions of the maximum peak height for H_2KA and n-hexacosane were shifted backwards slightly because of tailing of the front peak. For mixtures 5 and 6, H_2KA did not show a peak, but lay in a trough. At 217.3°, resolutions were approximately unity, and the peak height of H_2KA was overlapped by *ca.* 10 % each of K_3A and

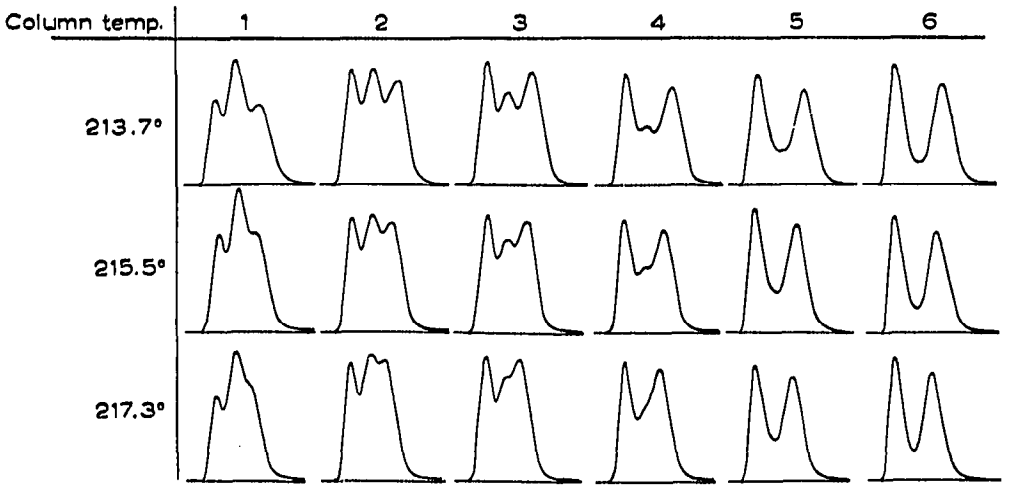

Fig. 2. Overlapping chromatograms of the mixtures of 5α-androst-3,11,17-trione, 3α,17β-dihydroxy-5α-androst-11-one and *n*-hexacosane at three resolutions.

n-hexacosane, where the peak maximum of H_2KA in mixture 2 was shifted only slightly but the displacement of the peak maximum of *n*-hexacosane was prominent. For mixture 4, in which the net peak height ratio of H_2KA to *n*-hexacosane was 0.5, the displacement for *n*-hexacosane had no perceptible effect on the quantitative analysis.

As seen in Table II, at the column temperature of 215.5°, a_{21} was zero, and accordingly the earlier equations are simplified to

$$H_1 = h_1 \tag{7}$$

$$H_2 = \frac{(h_2 - a_{12}h_1) - a_{32}(h_3 - a_{13}h_1)}{1 - a_{23}a_{32}} \tag{8}$$

$$H_3 = h_3 - a_{13}h_1 - a_{23} \cdot \frac{(h_2 - a_{12}h_1) - a_{32}(h_3 - a_{13}h_1)}{1 - a_{23}a_{32}} \tag{9}$$

At 213.7°, as the product of a_{23} and a_{32} is negligibly small, the above equations for H_2 and H_3 are further simplified to

$$H_2 = h_2 - a_{12}h_1 - a_{32}h_3 \tag{10}$$

$$H_3 = h_3 - a_{13}h_1 - a_{23}(h_2 - a_{12}h_1 - a_{32}h_1) \tag{11}$$

Analytical values of H_2KA are summarized in Table III and shown in Fig. 3. Even at lowest resolution, the quantitative values obtained were as accurate as the values at higher resolutions. Standard deviations at all three resolutions were less than 1%.

Analytical values for *n*-hexacosane (the last eluant) are shown in Table IV. For mixture 1, a higher content of H_2KA (the eluant in front of *n*-hexacosane) resulted in a low evaluation for *n*-hexacosane, but for mixtures 2–6 almost the same values were obtained at all three resolutions.

Quantitative values for mixtures 1 and 2 at 0.93 resolution shown in Tables III and IV were as accurate as those for the mixtures containing a lower H_2KA content.

These results suggest that the selection of the reference peak for retention time was appropriate. As we described in our previous paper[9], in which the higher of two unresolved peaks was used as a reference peak for retention times, analyses of peaks at unit resolution, where each peak height was comparable, showed a considerable error because of a displacement of the peak maximum of the reference from the true maximum. Therefore, for the analysis of a mixture at approximately unit resolution in which the amounts of components are comparable, the addition of a marker of known retention time, which elutes near the overlapping peaks, should enable an accurate value to be determined. Our concept proved to be valid for three overlapping peaks and could be applied with high accuracy even for a system composed of more than three overlapping peaks.

TABLE III

QUANTITATIVE ANALYTICAL VALUES (%) FOR 3α,17β-DIHYDROXY-5α-ANDROST-11-ONE (H_2KA) AT DIFFERENT RESOLUTIONS[a]

Resolution (H_2KA–C_{26})	*Mixture*					
	1	*2*	*3*	*4*	*5*	*6*
	Weight ratio, H_2KA/C_{24}					
	3.811	*2.540*	*1.905*	*1.270*	*0.635*	*0.257*
1.18	153.7 (0.61)	100.6 (0.62)	71.4 (0.71)	45.7 (0.61)	20.8 (0.57	7.2 (0.01)
1.08	151.7 (0.46)	98.7 (0.47)	(71.5) (0.40)	45.5 (0.02)	20.1 (0.01)	6.5 (0.01)
0.932	151.6 (0.93)	99.7 (0.61)	70.7 (0.51)	45.5 (0.03)	19.8 (0.41)	6.7 (0.02)

[a] Values in parentheses are standard deviations (%) for four determinations.

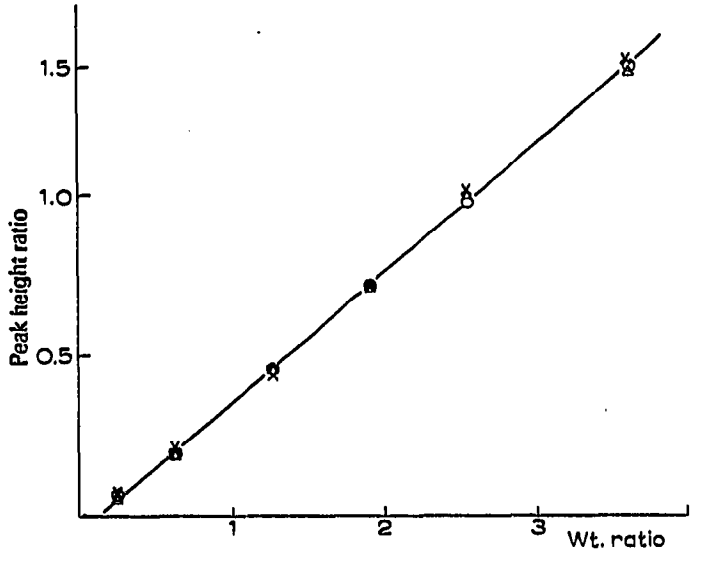

Fig. 3. Analytical values for 3α,17β-dihydroxy-5α-androst-11-one(H_2KA), with *n*-tetracosane as internal standard. Column temperatures (°C) and values of $R_{H_2KA-C_{24}}$, respectively: ×, 213.7, 1.18; ○, 215.5, 1.08; △, 217.3, 0.932.

TABLE IV

QUANTITATIVE ANALYTICAL VALUES (%) FOR *n*-HEXACOSANE AT DIFFERENT RESOLUTIONS[a]

Resolution (H_2KA–C_{26})	*Mixture*					
	1	*2*	*3*	*4*	*5*	*6*
	Weight ratio, C_{26}/H_2KA					
	0.5030	*0.7545*	*1.006*	*1.509*	*3.018*	*7.469*
1.18	91.4 (0.26)	92.7 (1.24)	94.1 (0.8)	93.7 (0.13)	93.3 (0.21)	92.6 (0.19)
1.08	92.5 (0.18)	93.7 (0.21)	95.4 (0.46)	94.9 (0.31)	94.9 (0.35)	94.3 (0.74)
0.93	92.1 (0.66)	93.8 (0.58)	96.0 (0.65)	95.9 (0.44)	96.8 (1.19)	94.3 (0.41)

[a] Values in parentheses are standard deviations (%) for four determinations.

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