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## LIQUID CRYSTALS AS THE STATIONARY PHASE IN GAS CHROMATOGRAPHY

### VI. APPLICATION OF THE ADSORPTION PHENOMENON TO THE ANALYSIS OF OVERLAPPING PEAKS

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#### SUMMARY

A method which provides a simple means for the determination of individual compounds which gave overlapping peaks on a gas chromatogram is described. It utilizes the adsorption property of the electric field applied liquid crystal column previously reported: under a constant electric field, the amounts of compounds adsorbed are independent of the amounts injected. The sample volume vs. response curves exhibit inflection points, theoretically predicted. Similar curves obtained experimentally for binary and ternary overlapping systems were exactly as predicted. Also, the amounts of individual compounds determined experimentally were in good agreement with those calculated from the theoretical curves.

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#### INTRODUCTION

In gas chromatography, there has been great progress in the development of high resolution columns, especially capillary columns. However, overlapping peaks are still encountered on chromatograms. In such cases, the utilization of other columns, a selective detector or other instrumental method has to be considered. However, alternative columns may result in overlapping of different peaks, and selective detection may be influenced by coexisting compounds. Although expensive gas chromatography-mass spectrometry (GC-MS) may be the best choice.

In this report, another way to identify and quantify the individual compounds of overlapping peaks is described. It is based on the utilization of an electric field applied liquid crystal (EF-LC) column, which has been investigated by the authors in recent years. When a direct current (d.c.) electric field is applied across a liquid crystal column, polar compounds are selectively adsorbed in it. The adsorption phenomena can generally be summarized as follows<sup>1-5</sup>:

(1) The amounts adsorbed,  $[A_d]$ , increase with increasing applied electric field,  $E$ .

(2) The larger the dielectric constant,  $\epsilon$ , of a solute, the greater is the amount adsorbed.

(3) The amount adsorbed is different from liquid crystal to liquid crystal.

(4) Isomers which have similar dielectric constants show different amounts of adsorption depending on the structure.

The relation between the amount adsorbed,  $[A_s]$  and these factors can be written as

$$[A_s] = k'(\epsilon - 1)f_s \cdot E$$

where  $k'$  is a constant characterizing the liquid crystal phase and solute functional group, and  $f_s$  is a structure term defined as the largest C-C bond number from the terminal carbon to the functional group.

(5) The amount adsorbed is dependent on the nature of the individual solute, but it is not affected by the overlapping of other compounds.

(6) Under a constant field strength, the amount adsorbed is constant, even if the amount injected is changed.

On the basis of these facts, a method is proposed to elucidate the individual compounds comprising an overlapping peak, and evaluated experimentally by using a few overlapping peaks. 4,4'-Di-*n*-amyloxyazoxybenzene was used as the liquid crystal for the EF-LC column. It was shown that there exists a good correlation between theoretical and experimental values.

The proposed method has various advantages: it is simple and could be performed by using only a short EF-LC column and a d.c. high voltage source; it could be used in combination with other analytical instruments as well as with various kinds of detector and it might be useful for preliminary sample treatment.

## THEORETICAL

In the proposed method, the calibration curves of the overlapping compounds need to be obtained beforehand. In other words, correction factors to detector responses ( $f_1, f_2, f_3, \dots$ ) should be available for converting peak areas into absolute amounts.

As mentioned in previous work<sup>1</sup>, the sample volume vs. peak area curve of a polar compound in the EF-LC system can be represented as in Fig. 1. The solid line (a) corresponds to the absence of an electric field, the dotted line (b) to the presence of an electric field. The lines are parallel and the distance between them, indicated as  $S_{ad}$ , corresponds to the amount adsorbed. When the responses to sample amounts  $S_1$  and  $S_2$  are  $A_1$  and  $A_2$  in the absence of an electric field and  $R_{E1}$  and  $R_{E2}$  in the presence of an electric field, the following relationships can be written:

$$A_1 = R_{E1} + S_{ad} \quad (1)$$

$$A_2 = R_{E2} + S_{ad} \quad (2)$$

Multiplying eqn. 1 by  $S_2/S_1 (= n)$  and subtracting eqn. 2 from it gives eqn. 3:

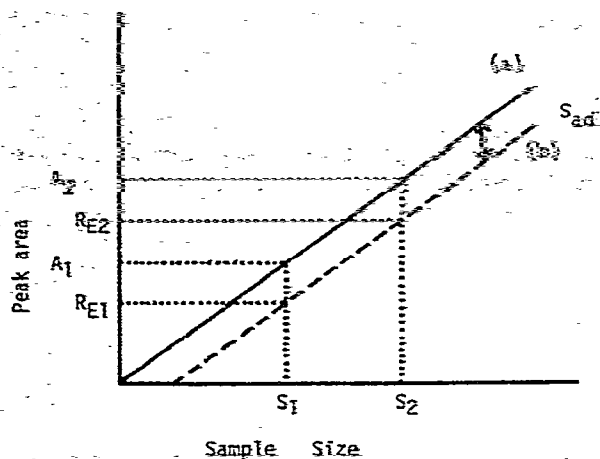


Fig. 1. Typical calibration curves of a polar compound: (a) without electric field; (b) with electric field.  $S$  = Sample size;  $R_E$  = peak area with electric field;  $S_{ad}$  = amount adsorbed;  $A$  = peak area without electric field.

$$nA_1 - A_2 = nR_{E1} - R_{E2} + (n - 1)S_{ad} \quad (3)$$

Since  $nS_1 = S_2$  and  $nA_1 - A_2 = 0$ , eqn. 3 can be written as:

$$S_{ad} = \frac{R_{E2} - nR_{E1}}{n - 1} \quad (4)$$

Eqn. 4 shows that  $S_{ad}$  can be obtained by using the responses at two different sample amounts in the presence of an electric field. Therefore, if there are enough adsorption data on individual compounds, or if the adsorption can be calculated from the molecular properties, identification of the adsorbed compound may be possible.

Next, subtracting eqn. 1 from eqn. 2:

$$A_2 - A_1 = R_{E2} - R_{E1} \quad (5)$$

Assuming the compound under study is known, and its calibration curve is available, the absolute amount in  $(S_2 - S_1)$  can be obtained from  $(R_{E2} - R_{E1})$  by using the calibration curves. Then, dividing this by  $(S_2 - S_1)$ , the concentration in the sample can be obtained.

#### Multi-component sample

Let us proceed to overlapping peaks. In the case of a binary overlapping system, the relation between the volume of sample solution constant concentration and the response can be represented as in Fig. 2. Fig. 2a shows the imaginary relationship between two separate solutes, and Fig. 2b shows the overlapping case. It is clear from Fig. 2b that the line does not pass through the origin and it also has an inflection point. The solid lines for compounds A and B in the absence of an electric field in Fig. 2a can be expressed as before:

$$A_i = A_{Ei} + S_{ad}^A \quad (i = 1, 2, 3, \dots) \quad (6)$$

$$B_j = B_{Ej} + S_{ad}^B \quad (j = 1, 2, 3, \dots) \quad (7)$$

As illustrated in Fig. 2b and described before, using responses  $R_{E1}$  and  $R_{E2}$  to the sample amounts  $S_1$  and  $S_2 (= nS_1)$  in an electric field,  $S_{ad}^A$  and  $(A_2 - A_1)$  can be expressed as in eqns. 8 and 9:

$$S_{ad}^A = \frac{R_{E2} - nR_{E1}}{n - 1} \quad (8)$$

$$(A_2 - A_1) = R_{E2} - R_{E1} \quad (9)$$

For compound B, with responses  $R_{E3}$  and  $R_{E4}$  obtained at sample amounts  $S_3$  and  $S_4$ , we obtain:

$$R_{E3} = (A_3 - S_{ad}^A) + (B_3 - S_{ad}^B) \quad (10)$$

$$R_{E4} = (A_4 - S_{ad}^A) + (B_4 - S_{ad}^B) \quad (11)$$

Assume  $S_4 = mS_3$ , then  $mA_3 = A_4$  and  $mB_3 = B_4$ . Therefore,

$$R_{E4} - mR_{E3} = (m - 1)(S_{ad}^A + S_{ad}^B) \quad (12)$$

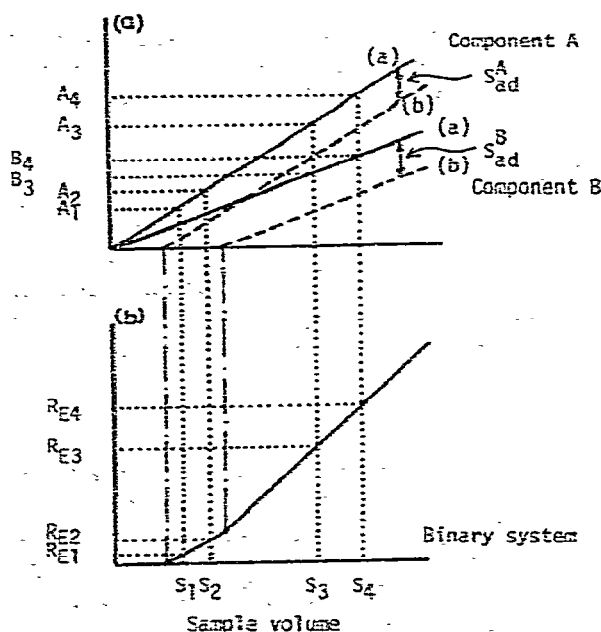


Fig. 2. Sample volume vs. response for a binary system.  $n \cdot S_1 = S_2$ .

which can be rewritten as:

$$S_{E2}^B = \frac{R_{E4} - mR_{E3}}{m - 1} - S_{E2}^A$$

Substituting  $S_{E2}^A$  by eqn. 8:

$$S_{E2}^B = \frac{R_{E4} - mR_{E3}}{m - 1} - \frac{R_{E2} - nR_{E1}}{n - 1} \quad (13)$$

From eqns. 10 and 11,

$$A_3 = S_3 \cdot \frac{R_{E2} - R_{E1}}{S_2 - S_1}$$

$$A_4 = S_4 \cdot \frac{R_{E2} - R_{E1}}{S_2 - S_1}$$

$$A_4 - A_3 = \frac{(S_4 - S_3)}{(S_2 - S_1)} \cdot (R_{E2} - R_{E1}) \quad (14)$$

then eqn. 14 can be rewritten as:

$$B_4 - B_3 = (R_{E4} - R_{E3}) - \frac{(S_4 - S_3)(R_{E2} - R_{E1})}{(S_2 - S_1)} \quad (15)$$

In a similar manner, cases where more than two components overlap can be treated. In Fig. 3, the sample volume vs. peak area relationships for ternary component systems is presented, and in Table I are given the equations for quantitation of individual compounds.

## EXPERIMENTAL

### Chemicals

The liquid crystal used in the EF-LC column was 4,4'-di-*n*-amyloxyazoxybenzene (nematic mesophase, 43–124°C). The stationary phase for the glass capillary separation column was squalane. *n*-Hexane, *n*-heptane, toluene, isovaleral, *n*-butyl acetate, isobutyl acetate, *sec*-butyl acetate, 2-hexanone, 3-methyl-2-pentanone and heptanal were all analytical grade.

Gas chromatography (GC) was carried out using a Shimadzu GC-6AMPr gas chromatograph with a flame ionization detector. The instrument was slightly modified in order to apply the electric field across the EF-LC column. The separation column was a whisker-walled glass capillary tube (25 m × 0.35 mm I.D.) coated with squalane. The EF-LC column (3 m × 0.35 mm I.D.), which was connected to the end of the separation column through shrinkable PTFE tubing, was prepared as described previously<sup>4</sup>. Inside it has a nickel wire (0.125 mm in diameter) and the outside

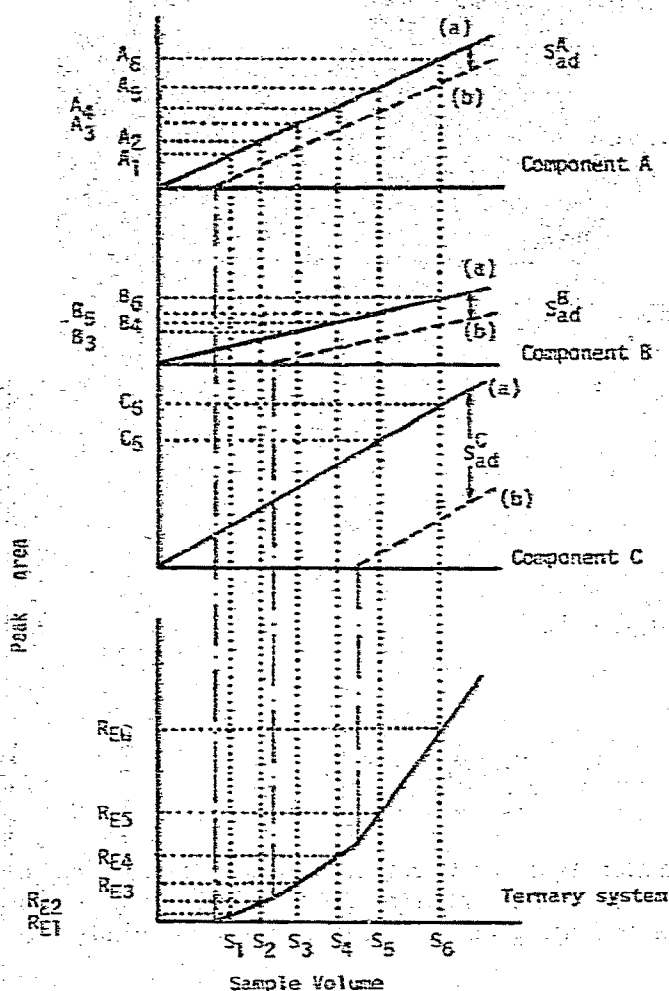


Fig. 3. Sample volume vs. response for a ternary system.  $n \cdot S_1 = S_2; m \cdot S_3 = S_4; k \cdot S_5 = S_6$ .

is coated with an electroconductive resin, Sealbest P-246 (Tokuriki Chemical, Kanagawa, Japan). An appropriate d.c. voltage was applied between the wire and resin.

For the d.c. generator, an Hamamatsu TV d.c. supplier (1.5 kV) for a photomultiplier was used at output voltages of 300 V or 500 V, which were applied to the EF-LC column. The carrier gas was pure grade nitrogen at a flow-rate of *ca.* 1 ml/min.

#### Sample solution

In order to construct individual calibration curves, 0.1% and 0.5% ether solutions of each compound were used. Overlapping peaks were achieved by use of the following combinations of compounds dissolved in diethyl ether:

- (i) *n*-hexane (0.1%)–isovaleral (0.2%)
- (ii) *n*-heptane (0.1%)–*sec.*-butyl acetate (0.2%)

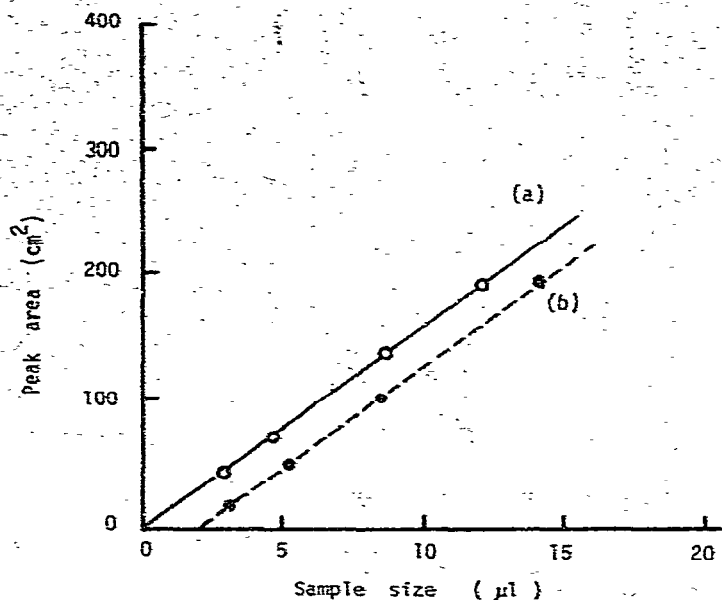


Fig. 4. Calibration curves for *n*-butyl acetate: (a) without electric field; (b) with electric field (500 V).

(iii) isobutyl acetate (0.1%)–3-methyl-2-pentanone (0.2%)

(iv) *n*-heptanal (0.1%)–*n*-butyl acetate (0.2%)

(v) toluene (0.1%)–2-hexanone (0.2%)–*n*-heptanal (0.1%)

The detector temperature and injection temperature were both 150°C, and the oven temperature was 90°C. When the GC conditions were changed, several hours were allowed before injection.

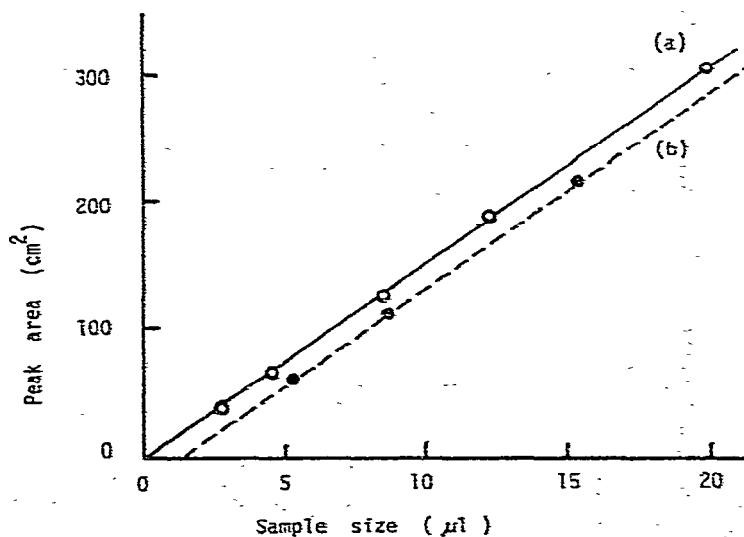


Fig. 5. Calibration curves for *sec.*-butyl acetate: (a) without electric field; (b) with electric field (500 V).

TABLE I  
EQUATIONS USED FOR IDENTIFICATION AND DETERMINATION

No. of components	Component	Eqn. for identification*	Eqn. for determination	Reference
1	A	$S_{nd}^A = \frac{R_{V2} - nR_{V1}}{n - 1}$	$A_2 - A_1 = R_{V2} - R_{V1}$	Figs. 1-3
2	B	$S_{nd}^B = \frac{R_{V4} - mR_{V3}}{m - 1} = \frac{R_{V2} - nR_{V1}}{n - 1}$	$B_4 - B_3 = R_{V4} - R_{V3}$	Figs. 2, 3
3	C	$S_{nd}^C = \frac{R_{V6} - kR_{V5}}{k - 1} = \frac{R_{V4} - mR_{V3}}{m - 1}$	$C_6 - C_5 = R_{V6} - R_{V5}$	Fig. 3

\*  $k = \frac{S_6}{S_5}$



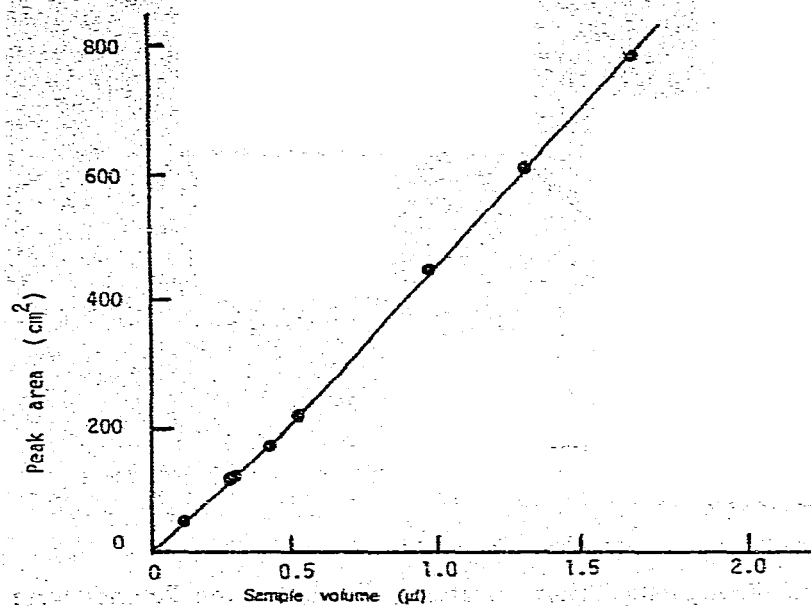


Fig. 6. Sample volume vs. peak area curve for *n*-heptane-*sec*-butyl acetate.

## RESULTS AND DISCUSSION

### *Calibration curves of standard samples*

Since toluene, heptane and hexane were not adsorbed in the EF-LC column, the calibration curves obtained with and without an electric field coincided with each

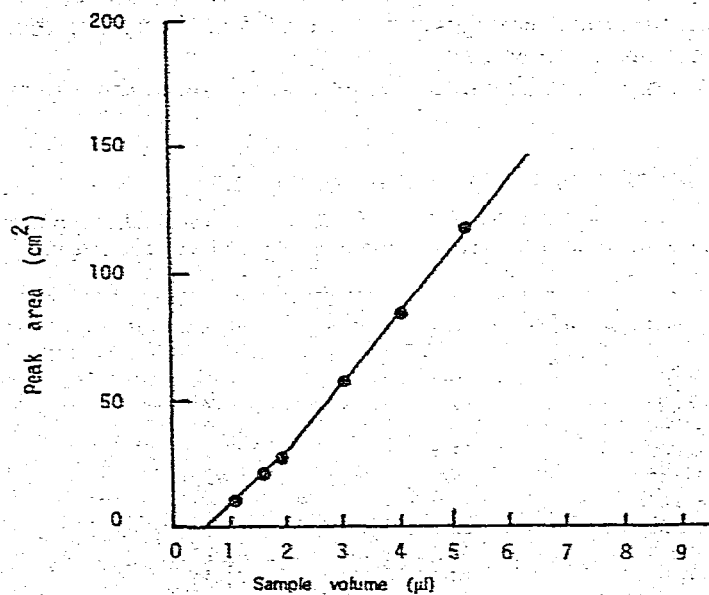


Fig. 7. Sample volume vs. peak area curve for *n*-butyl acetate-heptanal.

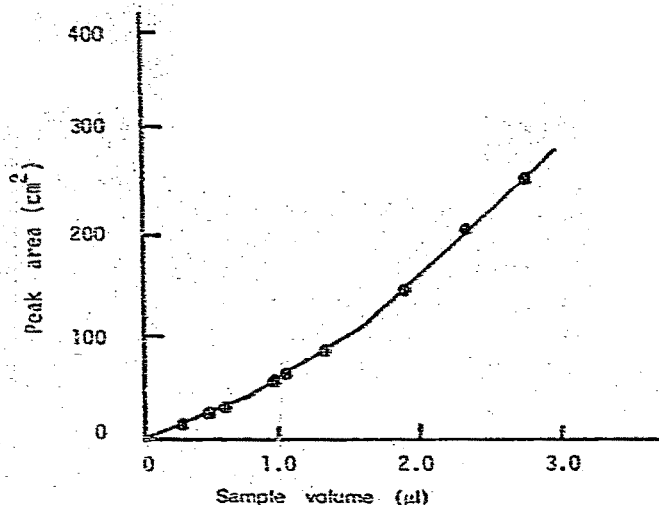


Fig. 8: Sample volume vs. peak area curve for toluene-2-hexanone-*n*-heptanal.

other and also passed through the origin. Acetates, aldehydes and ketones were adsorbed when an electric field was applied across the EF-LC column. Therefore, for each of them, two parallel lines were observed as shown in Figs. 4 and 5.

#### Overlapping peaks

The relationship between sample volume vs. response to the first sample (*n*-heptane-*n*-butyl acetate) is presented in Fig. 6. The line passes through the origin and has an inflection at about 0.5 μl. Because toluene is not adsorbed in the EF-LC column, the line passes through the origin. *sec*-Butyl acetate which was adsorbed completely in its lower concentration range emerges at above *ca.* 0.5 μl. Therefore, the responses from both compounds are summed above *ca.* 0.5 μl.

Fig. 7 shows the case of sample (iv). The line does not pass through the origin

TABLE II

#### ADSORBED AMOUNTS AND RETENTION TIMES OF STANDARD SAMPLES

Separation column, squalane (25 m × 0.35 mm I.D.); EF-LC column, 3 m × 0.35 mm I.D. Applied potential, 500 V d.c. Results given are amounts adsorbed (nmol), responses reduced (cm<sup>2</sup>) and retention times (min).

Compound	Amount adsorbed	Response reduced	Retention time
<i>n</i> -Hexane	0.000	0.0	2.62
<i>n</i> -Heptane	0.000	0.0	7.97
Toluene	0.000	0.0	13.94
<i>n</i> -Butyl acetate	0.205	34.5	13.77
Isobutyl acetate	0.185	27.6	9.37
<i>sec</i> -Butyl acetate	0.143	23.0	7.89
3-Methyl-2-pentanone	0.262	32.5	8.62
2-Hexanone	0.299	38.3	12.15
Isovaleral	0.302	24.5	2.90
Heptanal	0.421	32.1	13.65

TABLE III  
ANALYTICAL RESULTS FOR MULTI-COMPONENT SAMPLE

Sample No.	Retention time of overlapping peak (min)	Response reduced ( $\text{cm}^2$ )	Identification	Amount ( $\mu\text{g}/\mu\text{l}$ )				
				Found	Added			
1 A	2.73	0.0	<i>n</i> -Hexane	0.687	0.654			
				B	25	Isovaleral	1.71	1.77
2 A	7.94	0.0	<i>n</i> -Heptane	0.670	0.680			
				B	21	sec.-Butyl acetate	1.66	1.73
3 A	8.98	28	Isobutyl acetate	1.63	1.54			
				B	30	3-Methyl-2-pentanone	0.752	0.825
4 A	13.7	35	<i>n</i> -Butyl acetate	1.73	1.75			
				B	31	Heptanal	0.845	0.834
5 A	13.8	0.0	Toluene	0.854	0.862			
				B	37	2-Hexanone	1.79	1.66
				C	30	Heptanal	0.785	0.834

and has an inflection at about  $2.0 \mu\text{l}$ ; at this point both compounds are adsorbed in the EF-LC column.

Fig. 8 shows the case of sample v. Since the sample contains three compounds there are two inflections.

In Table II the retention times, amounts adsorbed and reduced responses of standard samples in an electric field of 500 V are presented. Using these data and the equations in Table I, five experimentally obtained relationships between sample volume and peak area, similar to those shown in Figs. 7 and 8, could be solved. Then, the  $S_{ad}$  values so obtained were compared with the values of the reduced responses in Table II. At the same time, the retention times were also compared. Coincidence of these data led to identification of individual compounds.

As for quantitation, the equations presented in Table I were again used. The responses per unit sample volume,  $1 \mu\text{l}$ , were calculated and then translated into concentrations by using the calibration curves for standard compounds.

The results obtained on the five examples run through the above procedure are summarized in Table III.

As the reduced responses were obtained graphically, accurate values for systems with more than two figures could not be obtained. It is, however, thought to be enough for the comparison of the calculated values with those of standard samples. The determined values were in good agreement with the theoretical values. In the case of sample (iii), the difference between the two values is rather large compared to the other cases. The reason for this is thought to be that the reduced peak areas are too small to afford accurate measurement.

From the results, it is clear that an EF-LC column could provide the means to identify and quantify individual compounds in overlapping peaks.

## CONCLUSION

When peak overlapping occurs in gas chromatography it is very hard to quantify the individual compounds.

A new method has been proposed for the quantitation of individual compounds in such peaks. By using the combination of a 4,4'-di-*n*-amyloxyazoxybenzene EF-LC column after a squalane analytical capillary column, the compounds in five test mixtures were successfully identified and measured.

The chromatographic system is simple and can easily be constructed by a small modification of a conventional gas chromatograph. The d.c. source for the application of the electric field across the EF-LC column can be replaced by dry batteries.

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