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STANDARD COLUMNS AND OPERATING CONDITIONS FOR DIVERSE ANALYSES BY GAS CHROMATOGRAPHY

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SUMMARY

The construction and characteristics of standard gas chromatographic columns suitable for use in the separation of diverse mixtures of volatile compounds are given. A method for determining the best isothermal period and temperature programme rate for the standard columns is described and the operating conditions that are suitable for use with a wide range of stationary phases separating all types of solutes are tabulated. Chromatograms indicating the relative performance of such columns are given and an example demonstrating the reproducibility of the standardised columns and operating conditions is included.

INTRODUCTION

Where a gas chromatograph is used for diverse applications ranging from the analysis of multi-component mixtures of wide boiling range to that of simple four or five component samples, much effort can be wasted in determining the optimum temperature programme to effect the required separation. Considerable time can also be spent in exploring the possibilities of different stationary phases when the required separation could have been obtained more quickly by using a standard stationary phase and choosing the correct column length. In the majority of gas chromatographic separations, special stationary phases are not usually necessary and if a number of columns of different lengths are available then two stationary phases are generally all that are needed. The two typical stationary phases that are normally used are a non-polar phase, *e.g.* Apiezon Grease, and a polar phase, *e.g.* PEG 20M. In order to standardize programming conditions, however, columns of known and reproducible resolving power must be available.

This paper describes a procedure for producing columns of different lengths having known and reproducible resolutions. An experimental method for determining the optimum initial isothermal period and programme rate is also described and the optimum programming conditions for each column length are given. Details of the construction and characteristics of seventeen columns are included, together with the respective optimum operating conditions that will give the best separation of the components of any type of sample without prior knowledge of its composition. The

only undefined variables left to the choice of the operator are the column length, the amplifier sensitivity and the chart speed.

COLUMN CONDITIONS

In order to operate columns under standard temperature programming conditions it is necessary to have a number of standard columns available that can be packed reproducibly to give a specified performance. If columns differ in performance, then they will require a different set of programming conditions to obtain optimum resolution and this would be very time consuming to determine.

The choice of column material depends on the solutes to be chromatographed. Metal columns are rugged, easily packed and may be used provided thermally labile or easily adsorbed solutes are not present. Glass columns are more fragile and in some instruments can be difficult to change. If thick-walled glass tube is used (1 mm wall thickness) such columns can be operated at gas pressures of up to 250 p.s.i. If mixtures are to be separated that may contain thermally labile substances, glass columns should be employed.

Theoretically the column diameter should be as small as possible to provide the maximum efficiency but in order to allow the column to be effectively packed and cope with adequate loads the minimum diameter is limited. McKENNA AND IDLEMAN[1], and SCOTT[2] have suggested optimum diameters of 4 and 2 mm, respectively. As the 4 mm diameter column was easier to pack reproducibly and can carry charges up to 50 μl this was chosen as the diameter of the standard columns.

The support must be as inert as possible and so acid-washed, silanised Celite was used. The effect of particle diameter on column efficiency has been studied by MELLOR[3], DESTY *et al.*[4], and CHESHIRE AND SCOTT[5], who show generally that the smaller the particle size, the higher the efficiency obtained from the column. However, supports of small diameter produce a high resistance to carrier gas flow and a compromise has to be reached with respect to the inlet pressures available to the column. The support sizes that were used for the standard columns are shown in Table I.

TABLE I

SUPPORT SIZES FOR THE STANDARD COLUMNS

Column length (ft.)	*Support size*
5	100–120 BS mesh, 152–124 μ
18	100–120 BS mesh, 152–124 μ
50	80–100 BS mesh, 185–152 μ

The support was coated in the normal manner, the stationary phase being dissolved in a suitable solvent. Care was taken to treat the support as gently as possible during the evaporation of the solvent and in subsequent handling, to prevent the production of "fines" by attrition. An even coating of stationary phase on the support is not essential as might be supposed. In Fig. 1 the HETP curve is shown for methyl and propyl acetates on a column packed with 12.5% w/w stationary phase on the support. One set of points is for the support coated directly with 12.5% w/w stationary phase, the other for a packing consisting of a mixture of equal quantities

of supports carrying 7.5%, 10.0%, 12.5%, 15.0%, and 17.5% w/w of stationary phase, respectively. It can be seen that both sets of points lie on the same curve, although one set is that from a column having a very wide range of film thickness and represents what might be considered a very poorly coated support.

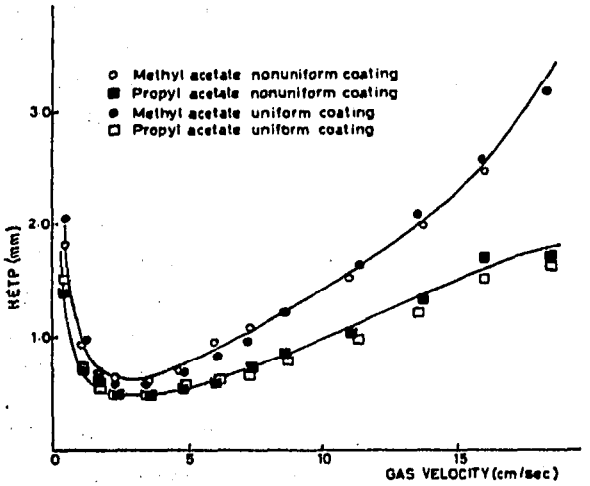

Fig. 1. HETP curves showing effect of homogeneity of support coating. Column temperature, 55°; support loading, 12.5% stationary phase.

The optimum quantity of stationary phase for maximum resolution and minimum analysis time varies with the type and molecular weight of the solute being separated, and the operating temperature. The optimum loading of stationary phase ranges from about 5 to 15% w/w, and to cope with mixtures of solutes having a wide range of molecular weights and polarities under temperature programming conditions, 10% w/w of stationary phase was taken as the standard support loading.

Each column was packed by attaching it to a pressurised funnel loaded with packing and applying a vacuum to the column exit. The packing was then transferred from the funnel into the column. A small wad of quartz wool at the end of the column prevented the packing from being lost down the vacuum line. After filling the column a pressure of 100 p.s.i. was applied to the inlet and the maximum packing density obtained by gentle tapping. The maximum column efficiency was then determined for a series of fatty acid methyl esters from C_{10} to C_{18} at 200° using the appropriate gas velocities for each column length as described later. A minimum efficiency of 600 plates/ft. was accepted; if this was not achieved the column was removed from the oven, and reattached to the vacuum and pressure lines. The column was again vibrated, more packing added and the efficiency measured again. This process was repeated until the required efficiency of 600 plates/ft. was achieved. It was found that all columns could eventually be made to give the required efficiency, but in some instances, particularly for the longer columns, it was found that the packing procedure had to be repeated as many as six times. The packing efficiencies obtained from a series of 5-ft., 18-ft., and 50-ft. columns are shown in Table II.

The relative lengths of the standard columns had to be determined by the increase in resolution required on changing from one column to another. Defining resolution as the ratio of the distance between two adjacent peaks to the average

TABLE II

EFFICIENCY OBTAINED FROM PEG COLUMNS OF DIFFERENT LENGTHS RECORDED IN PLATES/FOOT

	5-ft. column	*18-ft. column*	*50-ft. column*
1	625	660	659
2	640	600	600
3	640	600	570
4	690	711	
5	685	645	
6	680	744	
7	630	680	

peak width at the base, then the resolution is given by the following equation[6]:

$$R = \frac{(K_A - K_B)v_l n}{4(n)^{1/2}(v_g + Kv_l)}$$

where R = resolution,
K_A and K_B distribution coefficients of solutes A and B, respectively,
v_g = volume of gas per plate,
v_l = volume of stationary phase per plate,
n = efficiency in theoretical plates,

$$K = \frac{K_A + K_B}{2}$$

Thus

$$R = \frac{(K_A - K_B)v_l(n)^{1/2}}{4(v_g + Kv_l)}$$

If the same packing is employed and all columns are packed to give the same number of theoretical plates/ft. then K_A, K_B, v_l and v_g are all constant and n will be proportional to l, the column length. Thus, as the resolution R is proportional to $\sqrt{n}$, the resolution will also be proportional to $\sqrt{l}$.

Fig. 2 shows how the resolution of two peaks increases as the length of the column is increased. The improvement required in resolution by increasing the column length is very much a matter of arbitrary choice; for a significant increase in resolution it can be seen that the column length must be increased by a factor of 3 or 4 (*cf.* Fig. 2). As the standard columns were to be used with the Pye 104 gas chromatographs, the standard lengths were taken to suit the sizes available for these instruments, *viz.* 5 ft., 18 ft., and 50 ft. These columns give ratios of increase in length of 3.6 and 2.8, respectively, and thus the increase in resolution from column to column is 1.9 and 1.72, respectively.

Each column had to be used at a specific gas velocity and this was determined from the HETP curves of the respective columns. In Fig. 3, the HETP curves are shown for three standard columns packed with the standard packing carrying PEG 20M as the stationary phase. The curves were obtained by chromatographing a series of fatty acid methyl esters (C_{10}–C_{18}) at 200° at different gas velocities. The maximum

efficiency was obtained at each gas velocity by plotting efficiency against the retention time of each ester.

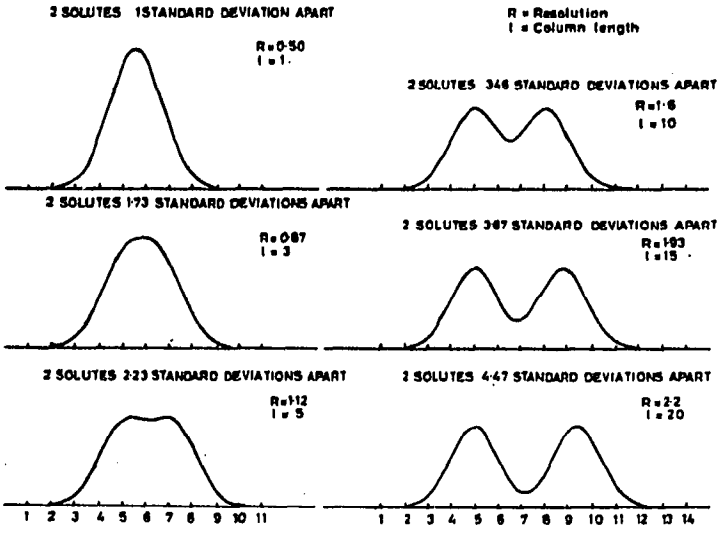

Fig. 2. Elution curves showing change in resolution with column length.

The efficiency increases at first and then levels to a constant value at about C_{14} to C_{18}. The constant value was taken as the maximum efficiency at that particular flow rate and from the series of such values the HETP was calculated at each gas velocity in the usual way[7]. It is seen from Fig. 3 that the gas velocities to be used for the 5-ft., 18-ft. and 50-ft. columns are 4.5, 3.5 and 3.0 cm/sec, respectively. These velocities correspond to values about half way between the optimum gas velocity and the optimum practical gas velocity for each column[8]. The decrease in standard gas velocity with column length reflects the increase in resistance to mass transfer affecting the HETP of the larger columns due to the higher column pressures reducing the diffusivity of the solute in the gas phase.

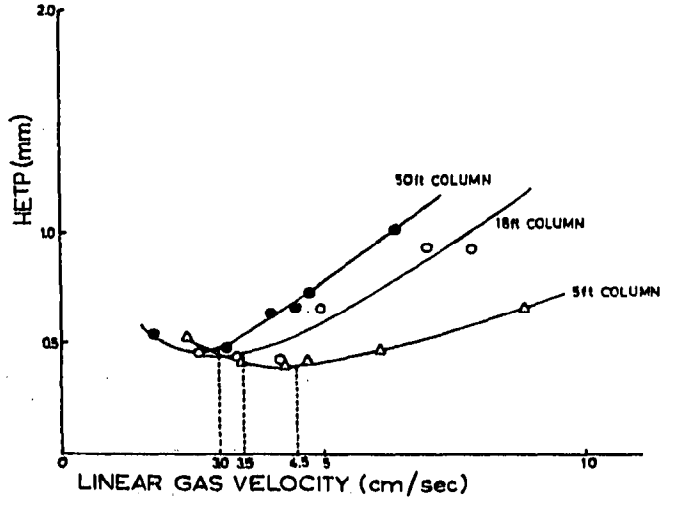

Fig. 3. HETP curves for standard columns, 5, 18 and 50 ft. long.

STANDARD CONDITIONS FOR TEMPERATURE PROGRAMMING

Any standard programming condition that would be applicable to the separation of diverse mixtures must be suitable for use with solutes and stationary phases of all polarities. It was therefore necessary to establish that the conditions would apply to solute types ranging from paraffins to alcohols, and stationary phases ranging from the non-polar, *e.g.* Apiezon Grease, to the polar, *e.g.* PEG 20M. For this reason, work on the 5-ft. column was carried out using both Apiezon Grease and PEG 20M as stationary phases and homologous series of hydrocarbons, esters and alcohols as solutes. Having established that the same optimum conditions applied for both Apiezon Grease and PEG 20M on the 5-ft. column, to conserve effort the optimum conditions for the 18-ft. column were determined using only PEG 20M as the stationary phase. However, the homologous series of paraffins, esters and alcohols were still used as solutes. The experiments carried out on the 5- and 18-ft. columns established that the same optimum programming conditions applied to all solutes and therefore the standard programming conditions were determined for the 50-ft. column using only PEG 20M as the stationary phase and the homologous series of esters as the solutes.

Initial isothermal period

The initial isothermal period had to be determined such that the maximum column resolution was obtained before the temperature programme was commenced. The homologous series of solutes were as follows: *n*-paraffins C_6–C_{20}; *n*-fatty acid methyl esters C_2–C_{18}; *n*-alcohols C_1–C_{16}. Samples of each of the homologous series were chromatographed on the 5-ft. column at approximately 50, 100, 150 and 200°, respectively, using both Apiezon Grease and PEG 20M as stationary phase. The normal alcohols were not chromatographed on the Apiezon Grease due to peak asymmetry. From the chromatograms obtained, the resolution of the column was calculated, for each carbon number of each homologous series and for each stationary phase, using the following equation:

$$R_n = \frac{y_{(n+1)} - y_n}{x_{(n+1)} + x_n}$$

where y_{n+1} is the retention distance of homologue $(n+1)$ from injection;

y_n is the retention distance of homologue (n) from injection;

x_{n+1} is the peak width of homologue $(n+1)$ taken at the points of inflexion;

x is the peak width of homologue (n) taken at the points of inflexion.

The graphs of resolution against retention time for each homologue of each series on each stationary phase for the 5-ft. column are shown in Fig. 4. It is seen that over the range of temperatures used the resolution between carbon numbers for the three series of solutes on both stationary phases at first increases with retention time and then tends to level to a constant value. Although the absolute values for the maximum resolution obtained for each series on each phase differ as they are different solute types, the maxima are achieved at about the same retention time.

The isothermal period for the 5-ft. column was taken as the mean value of the retention times where the resolution of the column reached 95% of its maximum

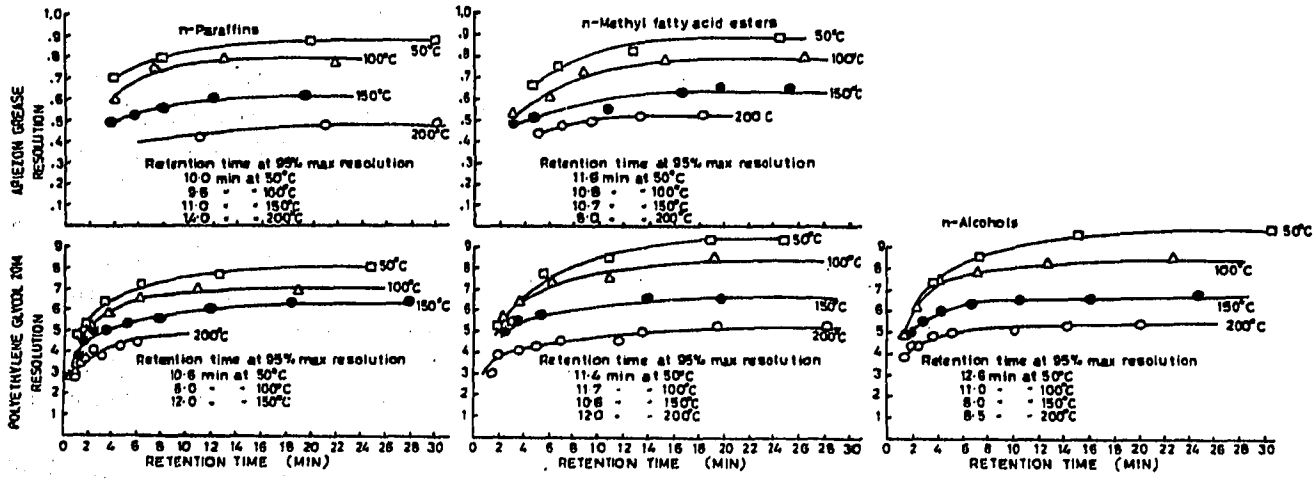

Fig. 4. Graphs of resolution against retention time for different solutes and stationary phases on a 5-ft. column.

value. The value of 95% maximum resolution is an arbitrary choice that attempts to compromise between adequate resolution and reasonable analysis time. (It is seen that to realise the extra 5% resolution would extend the isothermal period by about 50%.) On this basis the isothermal period for the 5-ft. column was taken as 11.0 min, the range between the different series and different stationary phases being min. 8 min–max. 14.0 min.

The same experiments were carried out on the 18-ft. column with PEG 20M as the stationary phase and the three homologous series of solutes. The 50-ft. PEG column was also examined using PEG 20M as the stationary phase at 200° but only the ester series was used as solutes. The initial isothermal periods were determined in the same way and found to be 39 min (min. 25 min–max. 57 min) and 60 min for the 18-ft. and 50-ft. columns, respectively. The graphs relating resolution and retention time for the 18-ft. and the 50-ft. columns are shown in Fig. 5.

It is seen that the intervals taken for the isothermal period are the averages of series of values that have a significant standard deviation. However, as the curves are fairly flat over this range, the corresponding standard deviation of the values for resolution about the mean interval that is taken for the isothermal period is much smaller, and constitutes only 2–3% of the maximum resolution. This justifies taking the intervals given above as standard isothermal periods for the column concerned.

The optimum programme rate

To determine the optimum programme rate, the homologous series of solutes were chromatographed on the different length columns at a series of different programme rates and the resolution between carbon numbers of each series was calculated. The details of the set of experiments which was carried out are shown in Table III. The results for the 18-ft. column packed with PEG as a stationary phase will be discussed first. The results obtained for this column, separating the three homologous series of alcohols, esters and alkanes, are shown in Fig. 6. The results for the normal alcohols show that the slower the programme rate the greater the resolution between each solute. At the higher programme rates there is a minimum in the resolution

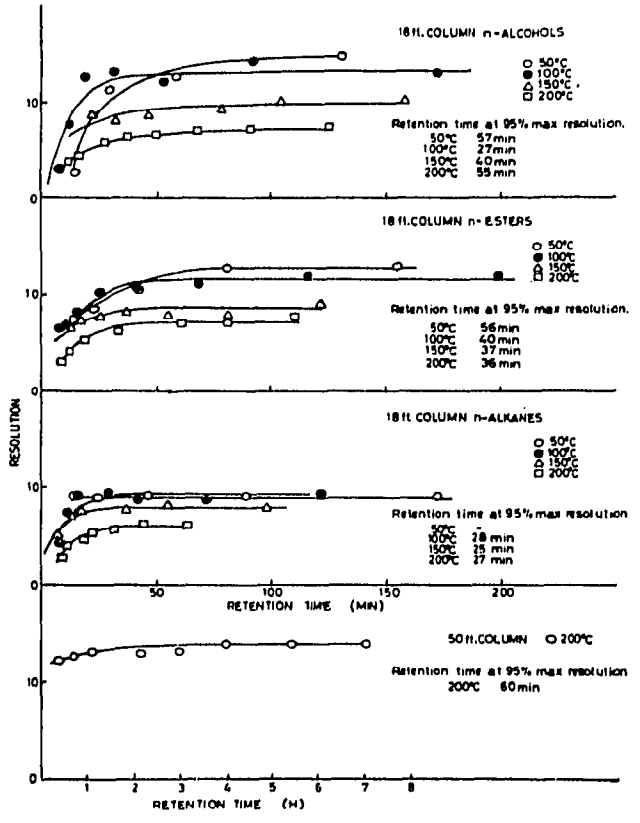

Fig. 5. Graph of resolution against retention time for different solutes on the 18- and 50-ft. columns.

TABLE III

EXPERIMENTAL CONDITIONS TO EXAMINE EFFECT OF PROGRAMME RATE ON THE RESOLUTION OBTAINED FROM EACH COLUMN

	5-ft. column	*18-ft. column*	*50-ft. column*
Stationary phase used	Apiezon L grease PEG 20M	PEG 20M	PEG 20M
Homologous series of solutes used	*n*-Alkanes C_6–C_{20} *n*-Methyl esters C_2–C_{18} *n*-Alcohols C_1–C_{16}	*n*-Alkanes C_6–C_{20} *n*-Methyl esters C_2–C_{18} *n*-Alcohols C_1–C_{16}	*n*-Methyl esters C_2–C_{18}
Initial isothermal period (min)	11	39	60
Temperature programme rate (°C/min)	0.5, 1, 2, 3, 4	0.25, 0.5, 1, 2, 3	0.15, 0.25, 0.5, 1, 2

that occurs between carbon numbers 10 and 12. This is due to the fact that the rapid rise in temperature has reduced the separation ratios to a greater extent than it has reduced the peak width. When the maximum temperature has been reached, however, the column is operating under isothermal conditions and the resolution increases again up to carbon number 15. The same situation occurs with the curves for the

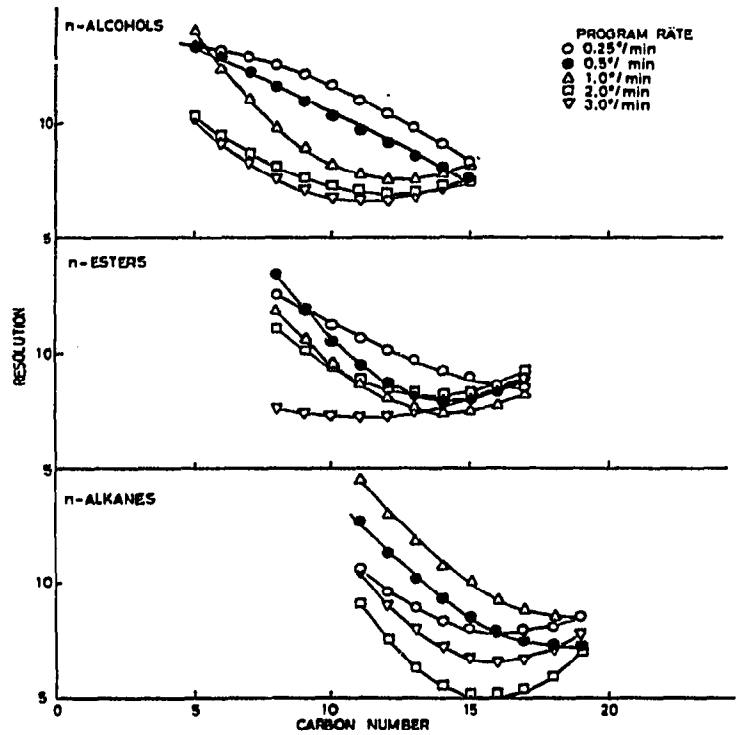

Fig. 6. Graph of resolution against carbon number for the 18-ft. standard column operated at different programme rates.

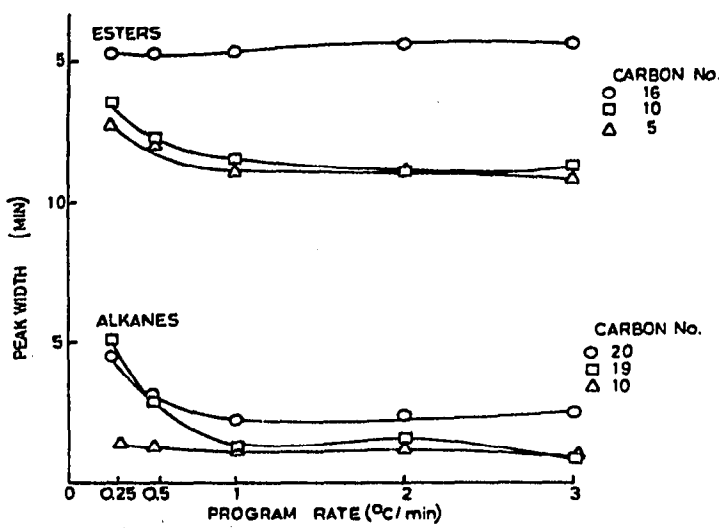

Fig. 7. Graph of peak width against programme rate for the 18-ft. standard column separating an homologous series of esters and *n*-paraffins.

esters. Again the higher the programme rate the higher the resolution and again minima occur in the resolution curves at the higher programme rates. For the normal alkanes, however, the situation is quite different. The maximum resolution is obtained at a programme rate of 1°/min and poorer resolution obtained at lower or higher programme rates than this. The reason for this can be seen from the results given in

Fig. 7, which are plots of peak width against programme rate for three members of the ester and alkane series. For the ester it is seen that as the programme rate increases the band width either remains constant or falls. In the case of alkanes, however, although the same is true for carbon numbers 10 and 20, the intermediate homologue 14 shows a maximum in the peak width at a programme rate of 2°/min. Now it was shown by SCOTT AND HAZELDEAN[8] that the resistance to mass transfer factor affecting the band width had a maximum value at a particular distribution coefficient or carbon number for a given homologous series. Furthermore this maximum in the resistance to mass transfer varied with the absolute temperature[9]. Thus for the alkanes we see this curious effect of a primary fall in band width as the programme rate increases and subsequent increase in band width. Because of this we have the optimum programme rate shown in Fig. 6 for the alkanes.

The best programme rate must always be a compromise between resolution and analysis time, so to determine this compromise the average resolution for the entire homologous series at each programme rate was plotted against programme rate for each column in Fig. 8.

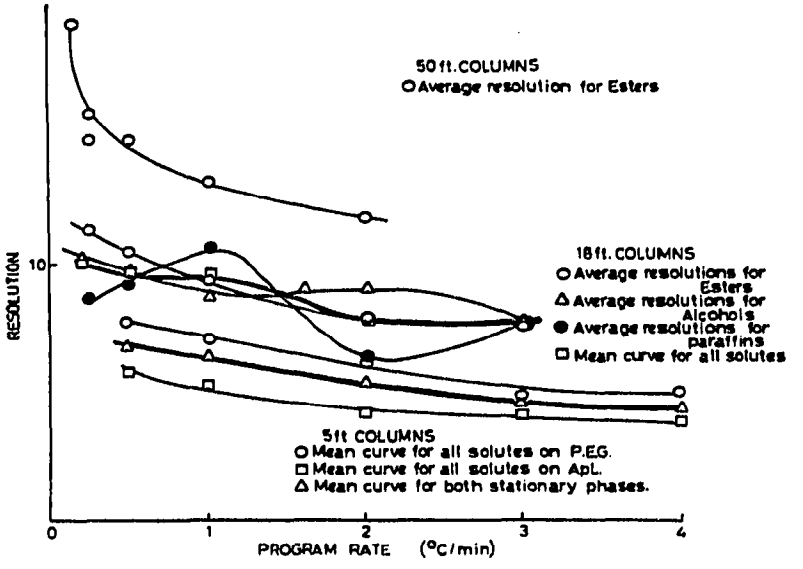

Fig. 8. Graph of resolution against programme rates for different length columns, different homologous series of solutes and different stationary phases.

Still concerning ourselves with the 18-ft. column we see that the average value for the resolution of the homologous series of esters falls continuously as the programme rate increases whereas the average value for the alkanes shows the S shape curve that follows logically from the results in Fig. 6. The heavy line for the 18-ft. column in Fig. 8 shows the mean curve obtained by averaging the three individual curves for each homologous series. As the columns have to cope with a complete range of solutes over a range of polarities, this mean curve will give the best programme rate for the 18-ft. column. It can be seen that this must be chosen at 1°/min as this gives 95% of the maximum resolution obtainable and that a further 5% increase in resolution can be obtained only at a sacrifice of four times the analysis time.

Considering now the 5-ft. column, the curves for each homologous series on

each stationary phase were averaged giving the light curves shown in Fig. 8. It is seen that the shape of these curves for both the polar stationary phase PEG and the non-polar stationary phase Apiezon L is the same and thus the optimum programme rate can be deduced from a mean of these two curves (shown as the heavy line for the 5-ft. column in Fig. 8). Arguing on the basis of the best resolution commensurate with a reasonable analysis time, it follows that the optimum programme rate will be 2°/min. Although operating at a programme rate of ½°/min would increase the resolution by about 8%, this would result in the analysis time being four times as long.

Finally, considering the 50-ft. column, which to economise on time was only examined using the methyl esters as solutes, the optimum programme rate is more of an arbitrary choice. The highest resolution was obtained for a programme rate of 0.15°/min but this resulted in an analysis time of nearly 26 h. However, to obtain as high a resolution as possible commensurate with reasonable analysis time a ¼°/min programme rate was chosen as the standard.

CONCLUSION

The results indicate that for the analysis of diverse mixtures the standard columns and operating conditions shown in Table IV should be used. These conditions have been used with six chromatographs over a period of a year and found to significantly reduce the time spent on chromatographic analysis and to provide more reliable

TABLE IV

CHARACTERISTICS OF STANDARD COLUMNS AND OPERATING CONDITIONS

	5-ft. column	*18-ft. column*	*50-ft. column*
Column diameter (mm)	4	4	4
Support particle size (BS mesh)	100–120	100–120	80–100
Stationary phase loading (%)	10	10	10
Minimum packing efficiency (plates/ft.)	600	600	600
Gas velocity (cm/sec)	4.5	3.5	3.0
Initial isothermal period (min)	11	39	60
Temperature programme rate (°C/min)	2	1	0.25

results from the point of view of reproducibility. During this period a wide variety of samples have been analysed and for no sample was it found necessary to deviate in any way from the standard conditions to obtain satisfactory results. An example of the results obtained using the standard columns for the separation of a complex essential oil, inchigrass oil, is shown in Fig. 9a, b and d. One sees the advantage of increasing lengths from 5 ft. to 18 ft. and then to 50 ft., the analysis time being 1 h 30 min, 4 h, and 13 h for each column, respectively. These columns can operate with loads ranging from 50 μg to 50 mg, maintaining approximately the same resolution. The reproducibility of a standard column is shown by comparing the chromatograms shown in Fig. 9b and c. These two chromatograms were obtained from two different standard 18-ft. columns in two completely different Pye 104 gas chromatographs using the standard operating conditions. The reproducibility can be seen to

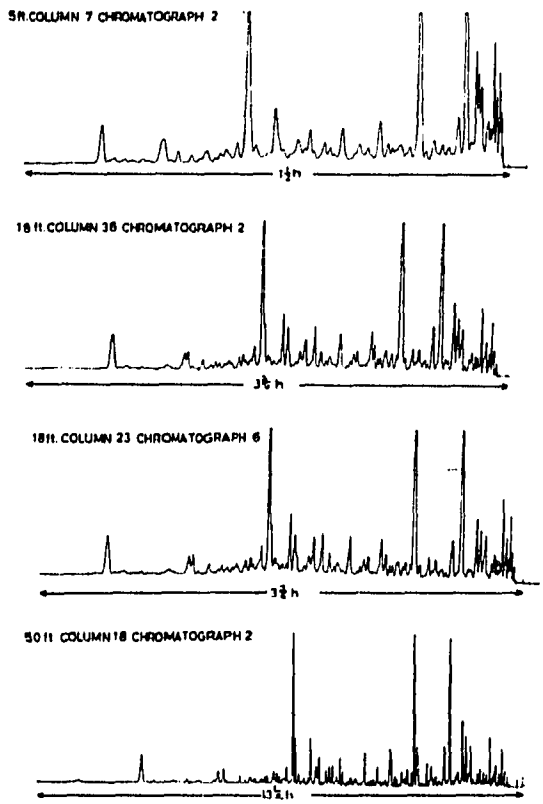

Fig. 9. Chromatograms of inchigrass oil on different standard columns.

be extremely good. This ease of reproducing identical chromatograms from the same sample with different instruments has been a great help when subsequent to preliminary analysis, the mass spectra or infrared spectra are required from specific solute peaks. With the use of standard columns and operating conditions the peaks of interest can be easily and reliably picked out even though run on another instrument in another laboratory.

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