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COMPREHENSIVE, STANDARDIZED QUALITY TEST FOR GLASS CAPILLARY COLUMNS

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SUMMARY

A quality test procedure for glass capillary columns is described that offers the following information: adsorption of hydroxyl function, adsorption of aldehyde function (an entirely independent characteristic), separation efficiency, acid–base behaviour and film thickness. This is the basic information needed for evaluating a column and for deciding the specific purpose to which the column can be applied. Important further progress consists in the way this information is obtained. A single run is needed, *i.e.*, no repeated runs are necessary in order to find optimal conditions. One test mixture produces the indicated information for all liquid phases. The standardization of conditions is such that the characteristics of columns with different liquid phases can be compared directly. Finally, the information is quantitative, which greatly increases its usefulness. Only quantitative information has been able to show that the common qualitative tests, *e.g.*, for acid–base behaviour, have often produced misleading interpretations. Exact directions for the test procedure and several practical examples are presented.

INTRODUCTION

The most fundamental problem in capillary column gas chromatography (GC) is the range of applications of the technique, and we must consider what types of sample will be analyzed on glass capillary columns in the future. The situation can be illustrated by an example. Approximately 5% of the organic matter in sewage effluents is analyzed by GC at present, in spite of the fact that much of the remaining 95% can be vaporized sufficiently and should consequently be amenable to GC. The reason why it is not amenable is well known: adsorption. We can, however, define the reason in less euphemistic terms: the weakness of the columns.

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There is evidence that much of this weakness can be overcome and this is the most fruitful area for study. For instance, it will be far more fruitful than the continuing discussion about how to increase further the reproducibility of the determination of separation efficiency. What is badly needed is a better understanding of adsorption processes and ways of controlling them. At present we have to develop the columns empirically.

Column development must be based on quality tests, and our knowledge of column characteristics cannot be better than the knowledge acquired from the tests. The test procedure described in this paper is practical and time saving on the one hand, and produces a wide range of information on the other. Interest in such a procedure is probably increasing now because of the increasing interest of column users in preparing their own columns, which offers the opportunity of optimizing the column characteristics for a particular analytical purpose. Again, this optimization calls for an informative quality test.

A NEW TEST: WHAT IT SHOULD TELL AND HOW IT SHOULD WORK

For over 10 years, an increasing number of column tests have accumulated in our laboratory. Even if some could be modified, the situation was so unsatisfactory that we decided to revise our testing fundamentally. The main changes were related to the following five requirements:

(1) the test should consist of a single chromatographic run;

(2) the test mixture should contain all of the many components necessary to give all of the basic information required;

(3) the same test should be applicable to all liquid phases;

(4) some quantitative aspects should be included;

(5) conditions should be standardized so as to make test results comparable.

Character of information

In the early development of capillary column GC, the most important quality was separation efficiency. Today, separation efficiency is only one of many pieces of information required from the test and is often not the most important. For all other information concerning different types of adsorption, a large number of compounds have to be added to the test mixture. Moreover, these components should be eluted sufficiently far from each other for mutual interaction to be avoided as much as possible. (It will be the purpose of a separate paper to show how substances, the retention differences of which should ensure perfect separation, may influence each other.)

The information provided by the test can no longer be expressed in terms as simple as "good" or "bad". Evaluation of differently prepared columns should reveal positive and negative aspects, making a column more suitable for one purpose than for another.

In the future, several components of the test as proposed under *Adsorption* can be replaced with even more specific ones. Other substances will be added with an increasing understanding of adsorption processes. To allow such adjustment, the test has to be designed flexibly.

Quantitative aspects

Quantitative aspects of capillary column GC have been neglected for many years. Well shaped peaks and a high separation efficiency were the prevailing criteria for column quality. Tests in use so far do not give any indication of whether or not a peak shows the full area required (or if the compound is 100% eluted from the column). It happened too often that even perfectly shaped peaks were far too small in terms of area owing to neglect of irreversible adsorption of the sample material. Quantitative work with such columns is obviously almost impossible.

Standardization

Adsorption tests are heavily dependent on the chromatographic conditions. Chromatograms are only comparable if standardized conditions are maintained.

As Fig. 1 shows, there are several ways of producing a well shaped peak of an adsorbing compound on a poor column. The adsorption of 1-octanol under different conditions is shown. Although the column is very active, thus strongly adsorbing polar material, it is still possible to produce a perfectly shaped peak for 1-octanol. However, the peak shows an insufficient area, indicating irreversible ad-

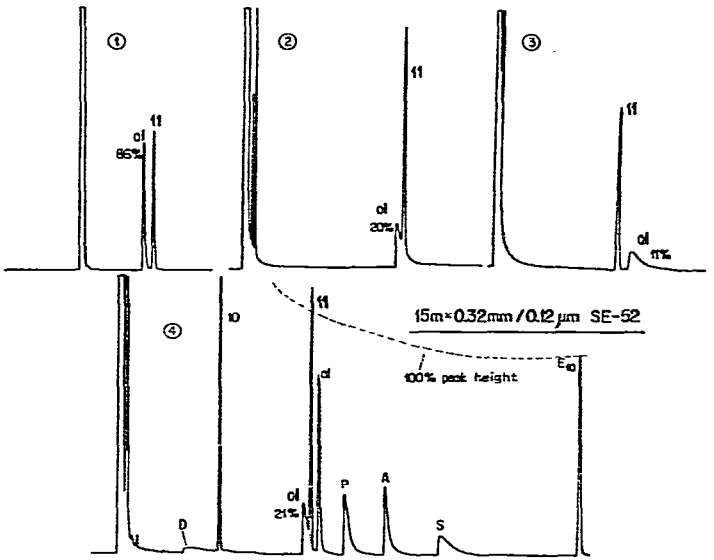

Fig. 1. Adsorption tests on a very poor capillary column, run within the same hour. Test compound: 1-octanol (ol). Undecane (11) was added in an amount to give the same peak area if no 1-octanol is lost. Chromatograms (1)–(3) were obtained isothermally. By choosing appropriate conditions, almost any peak shape desired can be produced from the column. In (1) the 1-octanol peak is almost perfectly shaped. Only the peak size (86% of the theoretical peak height) indicated some adsorption. For (1) forced conditions to favour the alcohol peak were used: 0.23 atm (H_2), 105°, attenuation $\times 128$, $k' = 1$ for the ol. In (2), conditions are close to standard: 0.35 atm, 50°, $\times 8$, $k' = 13$ (chart speed not the same for all chromatograms). In (3), conditions are excessively unfavourable for ol:0.6 atm, 40°, $\times 2$, $k' = 30$. Chromatogram (4) shows the new standardized test (adsorption related section of the chromatogram —compare with Fig. 10; for codes for other test substances see Table IV or Fig. 7): 0.35 atm (30 sec dead time), temperature programmed at 3.3°/min from 40°, attenuation $\times 8$ (using the full capacity of the column but without overloading as this would decrease the separation efficiency).

sorption. Under standard conditions (given by the optimal separation efficiency), the peak is considerably distorted and under more drastic conditions it tends to vanish. As almost any peak desired can be produced, the test is meaningful only if standardized and practice-oriented conditions are used. We propose to choose the conditions such that maximal separation efficiency is obtained (forbidding manipulations such as overloading, applying excessively high temperatures and very low retentions).

Aspects neglected by the proposed test

The test proposed here was developed pragmatically. It allows us to find the fundamental properties of a column in order to decide the purpose for which it can be used. For special purposes more specific tests are required. The basic test is not intended to provide data suitable for theoretical considerations or for deducing characteristics such as those described by Cram *et al.*[1].

WHY A TEMPERATURE-PROGRAMMED TEST?

The only way to meet all of the requirements stated above is to run the test under temperature-programmed conditions. Up to now, classical tests have always been run isothermally, except for theoretical work. We do not see any important advantages of isothermal test runs, whereas several strong arguments favour temperature-programmed tests, as outlined below.

Temperature adjustment

In temperature-programmed runs, the necessity of finding the proper temperature for the elution of a compound is eliminated. This eliminates the search by trial and error for the optimal conditions for, *e.g.*, a determination of TZ (Trennzahl, *i.e.*, separation number), which requires temperature optimization for each individual liquid phase and film thickness.

Number of test substances

For temperature-programmed runs, a relatively large number of test substances can be selected in such a way that the peaks become reasonably distributed over the whole chromatogram.

Comparable retention

Adsorption tests by isothermal runs will show the first peaks to be eluted under better conditions than the more retained peaks. Hence it is difficult to obtain comparable information. Temperature-programmed runs provide similar retentions for all components of the mixture, yielding results that can be compared within the chromatogram and (provided that the rate of temperature programming and flow-rate are standardized) also with tests from other columns.

Standardization

Temperature-programmed runs leave three variables to be chosen: carrier gas flow-rate, temperature programming rate and amount of test compound injected. The first two can be standardized (see below). The amount of test substance should not

be fixed as it is more practical to adjust it to the capacity of the column. This favours the injection of the maximal amount that does not decrease the separation efficiency (due to peak asymmetry) but gives the best adsorption characteristics.

One test mixture for all phases

The sequence of peaks of components with different polarities is dependent on the characteristics of the stationary phase. The mixture can be eluted in one run from all types of columns only if the temperature is programmed.

Rapid quantitative interpretation of chromatograms

Temperature-programmed runs elute peaks with an approximately constant width. If a mixture contains all components in an amount corrected by response factors to show the same peak area, then peak heights can be used for measuring areas. Hence a roughly quantitative interpretation is very rapid.

Independence of technique and equipment

In a temperature-programmed test, all reasonably retained components of a mixture are cold-trapped in the first part of the capillary immediately after injection. Therefore, an unsuitable injection technique does not cause peak broadening. This means that the separation efficiency is independent of the injection technique, *i.e.*, b_0 (according to Kaiser[2]) need not to be considered.

Of similar importance is the elimination of most of the effects of impurities (*e.g.*, septum particles) in the injector: adsorbing test components are retained on those spots and reach the column with a delay. This causes peak tailing in isothermal runs, which can hardly be distinguished from tailing caused by adsorption on the column. By temperature programming, the band is re-concentrated at the capillary inlet, eliminating this artifact.

Film thickness

The elution temperature of one of the peaks is used to measure film thickness (according to a calibration) (see below).

INFORMATION OFFERED BY THE NEW TEST

Separation efficiency

Several methods and quantities have been proposed for measuring separation efficiency. A good summary was given by Ettre[3,4]. Kaiser introduced a new set of "real" values[5,6]. For our practical work, we still prefer the TZ values (Trennzahl according to Kaiser). TZ values are of practical value as they yield, from two peak widths, and without the need for constant temperature, the separation efficiency directly in terms of how well two peaks are separated under experimentally meaningful conditions.

According to the classical method, TZ were measured with two neighbouring *n*-alkanes under isothermal conditions. As the results depend on the retention of the peaks, several injections were necessary in order to find the optimum.

The choice of alkanes for TZ measurements is related to the period when only hydrocarbons were eluted as symmetrical peaks. Today, this restriction is no

TABLE I

COMPARISON OF TZ VALUES OBTAINED ISOTHERMALLY AND BY TEMPERATURE PROGRAMMING

Comparison of TZ of methyl esters with those of alkanes.

Stationary phase	*Film thickness (μm)*	*Length (m) and I.D. (mm) of column*	$TZ_{prog.}$ (E_{10}/E_{11})	$TZ_{prog.}$ (E_{11}/E_{12})	$TZ_{prog.}$ *(average esters)*	$TZ_{isoth.}$ (C_{13}/C_{14})	$TZ_{isoth.}$ (E_{10}/E_{11})	$\frac{TZ_{isoth.}\ (C_{13}/C_{14})}{TZ_{prog.}\ \text{(average esters)}}$	$\frac{TZ_{isoth.}\ (C_{10}/E_{11})}{TZ_{prog.}\ (E_{10}/E_{11})}$
Pluronic L-64	0.15	20 × 0.30	33.8	32.5	33.2	32.6	33.7	0.97	1.00
Pluronic L-121	0.23	20 × 0.30	29	27.3	28.2	28.3	27.6	1.00	0.95
Pluronic L-101	0.15	20 × 0.30	32.5	31.4	32.0	34.0	31.3	1.06	0.96
OV-1	0.23	15 × 0.30	32.1	29.5	30.8	31.5	31.8	0.99	1.02
Pluronic L-64	0.18	20 × 0.29	33.4	31.0	32.2	36.0	33.3	1.12	1.00
Carbowax 600	0.28	15 × 0.30	26.6	25.1	25.8	25.8	27.0	1.00	1.02
Pluronic L-101	0.11	40 × 0.30	45.5	39.6	42.6	44.0	40.2	1.03	0.97
Pluronic L-64	0.15	20 × 0.31	29.6	29.0	29.3	30.6	32.6	1.04	1.00
SE-52	0.11	50 × 0.32	56.4	53.0	54.7	54.5	51.6	1.00	0.91
Carbowax 1000	0.18	45 × 0.31	43.1	41.0	42.1	43.7	44.0	1.04	1.02

longer justified. One of the problems of using alkanes is caused by their very different retentions on different stationary phases. As most workers tended to measure TZ values at about 70°, they had to use different pairs of alkanes for different stationary phases (C_{11}/C_{12} up to C_{17}/C_{18}). However, this produces results that are difficult to compare. The large changes in retention are related to changes in capacity for alkanes on stationary phases of different polarity. Carbowax columns with film thickness of 0.1 μm and less are almost inevitably overloaded at flame-ionization detector (FID) sensitivities, as their capacity for alkanes is well below 1 ng.

On searching for homologous compounds that have the most similar partition coefficients (and therefore the most similar retentions and capacities) in different kinds of stationary phases, we studied fatty acid methyl esters[7].

If TZ values are measured isothermally, there is only one pair of homologues at the optimal retention. In temperature-programmed runs, several values can be obtained by adding more than two homologous compounds. We chose three esters, providing two TZ values: the methyl esters of C_{10}, C_{11}, and C_{12} carboxylic acids (E_{10}, E_{11}, E_{12}, respectively). As all three peaks should have nearly the same width at half-height, irregularities can easily be detected.

As the relative difference in the molecular sizes of homologous pairs decreases with increasing molecular size, the first pair of methyl esters (E_{10}/E_{11}) provides a TZ value that is about 8% higher than that of the second pair (E_{11}/E_{12}). We normally use the average of the two values.

Comparison with classical TZ values

First we checked how the TZ values of E_{10}/E_{11} obtained in temperature-programmed runs compare with those obtained in isothermal runs. As the theoretical considerations are complex, we sought an experimental answer. Table I shows results from a number of columns. There is no significant difference between isothermal and temperature-programmed TZ values (the isothermal TZ values are often lower because the conditions have not been optimized carefully enough —a problem which is eliminated with temperature-programmed runs).

We then compared the ester TZ values obtained with classical values obtained isothermally with alkanes. Table I shows that the results with the alkane pair C_{13}/C_{14} are very near to or slightly higher than the temperature-programmed ester TZ values (as the average of the two values obtained from E_{10}/E_{11} and E_{11}/E_{12}).

All of the values in Table I were obtained with hydrogen as carrier gas. For helium the separation efficiencies of the capillaries were 5–10% higher.

We conclude that the ester TZ determination is both practical and reproducible. We emphasize that Kaiser's recent warning concerning the dependence of TZ on k' applies to non-optimal conditions. In our test, optimal conditions are ensured automatically.

Adsorption

Adsorption on capillary columns has usually been determined either by the tailing of a peak or by its extra retention (McReynolds data).

Adsorption tests have often been carried out with a "polarity mixture"[8] containing a hindered alcohol, a ketone and naphthalene together with some *n*-alkanes.

In recent years this mixture has been increasingly replaced with a single substance, normally a primary alcohol such as 1-octanol or 1-butanol[9].

We do not use retention data to determine adsorption. The column with the lowest retention for an alcohol (for a given liquid phase) is, as we found by experiment, not necessarily that with the least adsorption (peak distortion). Different types of support material (silica, sodium chloride, barium carbonate or barium sulphate) have specific polarities. Very thin film methylsilicone columns are much more polar than thick film columns[10,11]. On the other hand, polarity increases with increasing film thickness of very polar stationary phases (Silar 10 or Carbowaxes). Hence the increased retention of polar solutes implies a decreased influence of the support surface.

The shape of the peak is also not sufficient for detecting adsorption. We often observed peaks of almost perfect shape but with far too small an area. Tests based on distorted peaks (or even "peak shape parameters"[1]) neglect that in many instances part of the adsorption is not reversible, diminishing the amount of the component eluting from the column, and the worst of the different types of adsorption is missed.

There is little information available on the mechanism of adsorption. Adsorption sometimes causes (a) a broadened peak of Gaussian shape, (b) a tailing peak of more or less correct area, (c) a reasonably shaped peak of reduced area or even (d) a misshapen peak of correct area but drastically increased retention (Kováts indices >300), as shown in Fig. 8.

The measurement of peak height as a percentage of that expected for complete and undistorted elution is a very easy means of measuring adsorption. It covers all types of peak distortions which are relevant in practice: peak broadening, peak tailing, irreversible adsorption or degradation. To distinguish between reversible and irreversible adsorption, peak integration is necessary.

In order to illustrate the above, Fig. 2 shows the test chromatogram of a column experimenting with acidic supports.

Adsorption of polar solutes. We must consider reasonable tests for adsorption

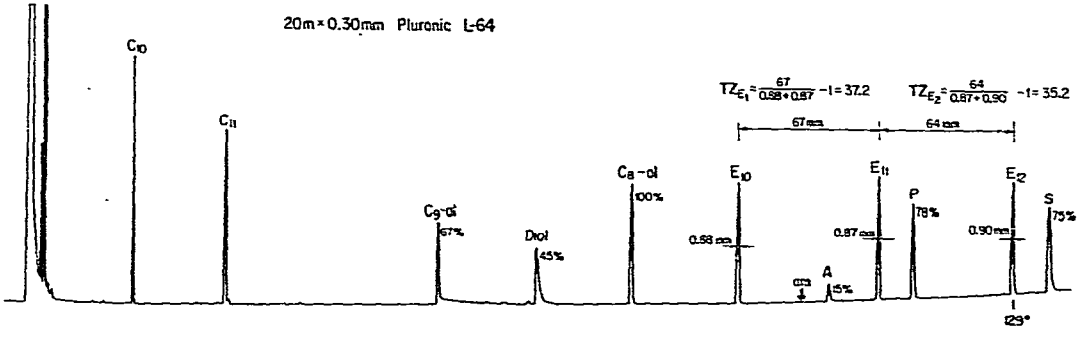

Fig. 2. Test chromatogram of a column which turned out to be a failure: $BaCO_3$ on HCl-leached Pyrex converted into $BaSO_4$. Liquid phase: Pluronic L-64, a polyethylene–propylene glycol copolymer[12]. Test after conditioning at 220° for one night (for codes for test substances see Fig. 7 or Table IV). The AP test indicates "acidic", but both A and P are adsorbed. This shows that there are some basic active sites left (besides more acidic sites). No am elutes, S elutes to only 75% due to the basic sites also active to P. Adsorption by hydrogen bonding is acceptable (not good). The al seems reasonable at first glance, but one third of the material is adsorbed. The separation efficiency is relatively high. The film thickness is 0.18 μm as E_{12} elutes at 129°.

phenomena caused by unknown mechanisms. Empirically, tests with an alcohol are very informative: alcohols are more sensitive to adsorption than most other functional groups (provided that they are acid–base inactive). A positive test with a primary alcohol normally means that a column shows little adsorption of other polar functional groups.

The adsorption of hydroxyl groups might be caused by a hydrogen bonding mechanism. Consequently, alcohols cannot really be regarded as independent of acid–base effects, which is in agreement with the work of Burns and Hawkes[13]. However, this dependence can be neglected for columns with support surfaces near to neutral, which are normally used.

An alcohol commonly used as a test compound is 2-propylcyclohexanol[8]. As adsorption on modern glass capillary columns is considerably reduced, more stringent tests should be used. We chose two different alcohols: 1-octanol (ol) (as used in many laboratories) and 2,3-butanediol (diol or D). The primary monoalcohol is appropriate for testing columns coated with a liquid phase without hydroxy groups in their structure (*e.g.*, silicones). Glycol-type stationary phases, however, normally show a 100% peak height with 1-octanol, *i.e.*, all of the material injected is eluted without adsorption. Therefore, the diol is more informative. Columns coated with non-polar silicone phases elute the diol at the front of the chromatogram, but even there with strong tailing. Columns producing 100% peaks for the diol are exceptional in our laboratory, even among those coated with Carbowaxes or Pluronics.

On extremely acidic columns, the diol normally shows improved peaks, whereas the peak height of the ol is often reduced by 10–20%. On basic columns, the diol is usually more affected than the ol.

The test of adsorption through hydrogen bonding is informative for other related functional groups. Carboxylic acids adsorb only slightly more strongly than 1-octanol on supports without basic sites (provided that the column is not so strongly acidic that it causes tailing of the alcohol). Primary amines form weaker hydrogen bonds than the primary alcohol and therefore elute better than 1-octanol from columns free of acidic sites.

Adsorption of aldehydes. Our test mixture contains *n*-nonanal (al) for investigating the adsorption of saturated aldehydes. This type of adsorption is independent of the test on hydrogen bonding. Aldehydes often broaden or tail on glycol phases, depending on unknown factors. As an example, two test chromatograms of PG 600 columns on barium sulphate and carbonate are shown in Fig. 3. In this instance, it appears as if barium sulphate would be the preferable support material. However, we observe perfect peaks of al on barium carbonate supports also.

Adsorption due to acid–base effects. A major drawback to the use of glass for capillary columns is its activity towards acids and bases. Glass is acidic, neutral or basic. The test for adsorption due to this activity is important as regards a decision on which column can be used for a particular type of sample. Normally a column is either suitable for acids or for bases, or very often for neither.

Acid–base adsorption cannot be tested by peak shape (as has often been reported in the literature). We have produced many columns that elute acidic or basic compounds as perfectly shaped peaks, but with an area only 30–50% of that expected. Chromatograms with perfectly shaped peaks of amines and phenols do not prove the inertness of a certain column to acids and bases. In our laboratory, such

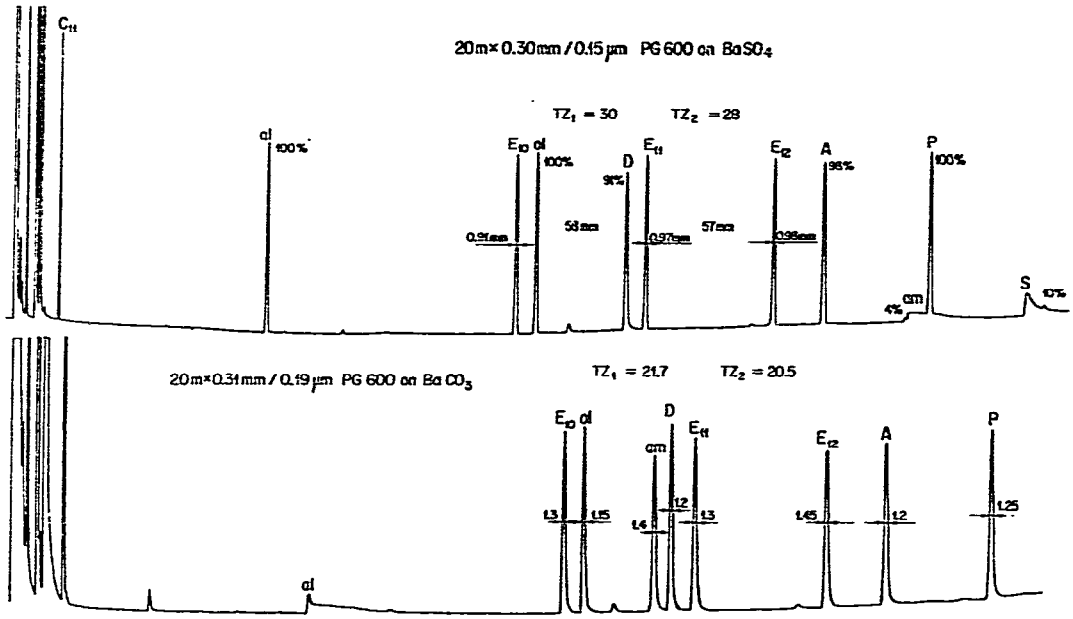

Fig. 3. Test chromatograms of two columns with the same stationary phase and very similar dimensions. According to the AP test, both columns are very near to neutral without any strongly acidic or basic sites. The first column elutes al as 100%. The second shows the al as a poor peak. The first column has an efficiency we consider normal. Adsorption due to hydrogen bonding is very low (D, 91%). Am and carboxylic acid elute badly. The second chromatogram is odd: all peaks are tailing and the TZ values are too low. In fact, there was a small droplet of bleeding material at the column end. After washing the droplet away, TZ was 27. The al was the same, adsorption of alcohols being extremely low (D, 100%), inert to bases (am, 100%) but very active to carboxylic acids (S, 0%) ($BaCO_3$).

columns usually produce 100% peaks for neither the acid nor the base. Well shaped peaks are then misleading because the columns cannot be used for the quantitative analysis of either acids or bases.

Our test mixture contains 2,6-dimethylaniline (DMA or simply A) and 2,6-dimethylphenol (DMP or P). By measuring peak heights, the major deficiency of the "AP-test"[14] could be eliminated: often, especially on nearly neutral columns, neither DMA nor DMP elutes as a 100% peak. Obviously these columns contain both acidic and basic sites. The original qualitative test would indicate either basic or acidic according to which of the two peaks is higher. It does not indicate, however, whether or not the column is suitable for the quantitation of acids or bases. The Pluronic L64 column in Fig. 1 may serve as an example.

In addition to the DMA–DMP pair, the test mixture contains another pair of compounds for a more stringent acid–base test, namely 2-ethylhexanoic acid (S) and dicyclohexylamine (am). As in DMA and DMP, the functional groups of these two substances are sterically hindered in order to avoid adsorption by hydrogen bonding (only acid–base effects are of interest).

Film thickness and retention

It is usually of interest to have an easy method for checking the film thickness

in dynamically coated columns. Dynamic coating can hardly be standardized to yield a predictable film thickness.

Another parameter of interest is the changes in retention of a column after prolonged use. Reduced retention sometimes means that the column has lost a considerable part of the liquid phase through bleeding, but more often it is caused by contraction of the film. Therefore, it is essential to have a fairly exact check of retention during the whole lifetime of a column.

Retention can easily be deduced from the elution temperature of a peak during a temperature-programmed run. Firstly, the retention of the substance used for this purpose should be little affected by polarity changes (which may occur during prolonged use of a column) or by the dependence of the polarity on factors other than the liquid phase (film thickness, support material) because this would complicate calibration and extrapolation as described below. Secondly, for practical reasons it is essential that the peak to be used is clearly within the temperature-programmed part of the chromatogram for all film thicknesses and stationary phases —this means that the peak elutes at least 50° above the injection temperature. The methyl ester E_{12} is suitable in both respects.

The elution temperature of E_{12} is reproducible to within 1° if standard conditions (carrier gas flow-rate and temperature programming rate) are kept within reasonable limits; in terms of film thickness, this means a variation of 5% or less. Quantification of film thickness requires calibration of all stationary phases of interest. Table II gives the elution temperature (standard elution temperatures) of E_{12} for columns coated with 0.15-μm films of liquid phase.

TABLE II

ELUTION TEMPERATURES OF E_{12} FROM 0.15-μm THICK FILMS

Standard elution temperatures.

Stationary phase	*Elution temperature (°C)*	*Stationary phase*	*Elution temperature (°C)*
OV-1	118	Pluronic L-64	124
SE-52	122	Pluronic F-68	118
SP-2125	123	Emulphor ON-870	125
OV-17	127	Ucon HB-5100	127
OV-225	117	Carbowax 400	103
SP-2300	118	Carbowax 600	107
OS-124	130	Carbowax 1000	108
Pluronic L-61	135	Carbowax 2000	110
Pluronic L-121	132	Carbowax 4000	110

Another calibration is needed for the extrapolation from the standard film thickness to the one of interest. Fig. 4 shows the dependence of elution temperature on film thickness, the film thickness being given on a logarithmic scale. The curves of film thickness *versus* elution temperature have similar but not identical slopes for different stationary phases, possibly because the films are not completely homogeneous, perhaps owing to polarity changes resulting from variation of the film thickness or viscosity with temperature. We assume that a factor of 2 in the film thickness changes the elution temperature by 14° (the measured values are 14° for SE-52, 13° for Pluronic L-64, 13.5° for Pluronic L-121 and 14.5° for Carbowax 4000).

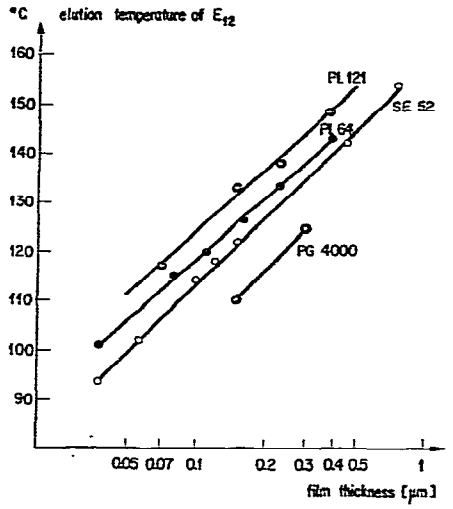

Fig. 4. Elution temperature of E_{12} (dodecanecarboxylic acid methyl ester) under standard conditions from statically coated columns of different film thickness (plotted on a logarithmic scale). The dependence of elution temperature on film thickness is shown for four stationary phases.

Fig. 5 demonstrates a means of estimating film thickness from the difference between the measured elution temperature and the standard elution temperature (0.15-μm film) given in Table II. The result obtained in this way has an accuracy of about $\pm 10\%$ for film thicknesses ranging from 0.08 to 0.3 μm.

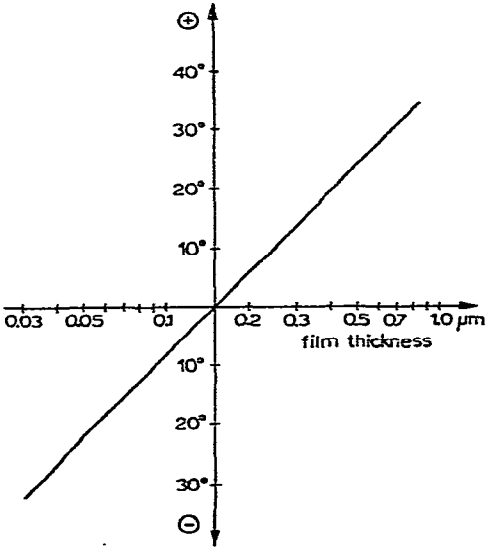

Fig. 5. Aid for estimating film thickness. The elution temperature of E_{12} measured for a column is compared with the standard elution temperature (for a column of film thickness 0.15 μm) given in Table II. The difference in these two temperatures is brought on to the vertical axis (positive differences in the upper half). The horizontal axis gives the film thickness with about a 10% standard deviation in the range 0.8–3 μm.

EXPERIMENTAL

Carrier gas flow-rate and temperature programme

Conditions for test runs, carrier gas flow-rate and temperature programme were optimized empirically to find the optimal TZ value.

According to theoretical considerations, it would be surprizing if the same conditions were optimal for all types of column. Therefore, a large number of columns were tested with different flow-rates and temperature programmes. These columns had different inner diameters (0.25–0.33 mm), different lengths (15–50 m), different liquid phases (SE-52, OV-17, Pluronic L-64, L-121 and L-61 and Carbowax 600 and 1000) and with film thicknesses between 0.07 and 2 μm. The optimal flow-rate and temperature programme were found to cover surprisingly wide ranges and to allow one to use the same conditions (which yield the best TZ value) for all of the columns being tested.

It proved best to optimize conditions by considering two parameters: ratio of carrier gas flow-rate to rate of temperature programming and the speed of the run. They can be considered to be independent of each other over a reasonably wide range of conditions. This must be explained further, although it is impossible to reproduce all of the data obtained during this work.

Fig. 6 shows the results for one of the columns tested. Separation efficiency (as an average of the two ester TZ values) is plotted against t_0, the dead time of methane at 25°. For every temperature programming rate, there is an optimal carrier gas flow-rate. This optimum is shifted to a higher value (*i.e.*, to a lower dead time, t_0) if the rate of temperature programming is increased.

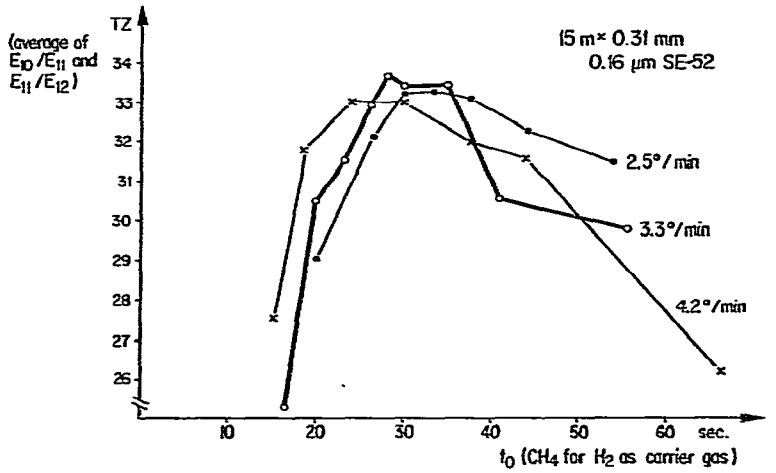

Fig. 6. TZ values measured at different carrier gas flow-rates (determined by dead time) and rates of temperature programming for one of the columns used to find optimal conditions for separation efficiency. These optimal conditions are used as standard conditions for the test. The plot shows three optima for the ratio of carrier gas flow-rate to temperature programming rate. A faster temperature programme requires a higher carrier gas flow-rate (*i.e.*, a shorter dead time). The three optima differ in the speed of the run. The quickest run (4.2°/min) yields a lower TZ value than the two slower runs, which can be considered equal. The standard conditions for the test are 3.3°/min for the temperature programme and a dead time of 30 sec if hydrogen is used as the carrier gas.

In the first step, the optimal ratio of carrier gas flow-rate to rate of temperature programming was determined. This ratio is also expressed by the optimal elution temperature of a given compound.

The second step was to vary the speed of the run by changing the flow-rate and temperature programming rate in proportion. As is known for the isothermal measurement of TZ values, efficiency increases with increasing retention (in our case with slower flow-rates and lower temperature programming rates). The TZ values reach a plateau that is dependent on the quality of the coating: a column with a high efficiency requires a higher retention (slower run) to yield the plateau value than a column of low efficiency. Hence the speed of the run had to be determined on the most efficient columns.

The standard deviations of TZ values are on the order of 5–7%. They are still lower than the deviations we obtained from isothermal runs, mainly because the conditions for the latter have not been optimized carefully enough.

The rules for finding the standard conditions for a given column are simple. As Table III shows, the dead time (measured with methane at room temperature) has to be 2 sec per metre length of the capillary if hydrogen is used as the carrier gas. The temperature programming rate can be deduced from 1°/min for a column of length 50 m. Helium is more viscous and requires slower runs than hydrogen: the length of a column in metres has to be multiplied by 3.5 in order to calculate the dead time in seconds. The temperature programming rate is 0.5°/min for a column length of 50 m.

TABLE III

STANDARD CONDITIONS FOR FLOW-RATE AND TEMPERATURE PROGRAMMING
Hydrogen and helium as carrier gases.

Column length (m)	*Hydrogen*		*Helium*	
	CH_4 elution (sec)	*Temperature programme (°C/min)*	*CH_4 elution (sec)*	*Temperature programme (°C/min)*
10	20	5.0	35	2.5
15	30	3.3	53	1.65
20	40	2.5	70	1.25
30	60	1.67	105	0.84
40	80	1.25	140	0.63
50	100	1.0	175	0.5

As we consider that nitrogen should not be used as the carrier gas in work with capillary columns, we did not determine suitable conditions for this gas. However, runs should be approximately three times slower in terms of flow-rate and temperature programming rate than those with hydrogen as the carrier gas.

In order to obtain a correct dead time for columns with films thicker than about 0.7 μm, t_0 was measured at 100° (methane is considerably retained at 25°). As the viscosities of the carrier gases are increased at this temperature, we corrected the dead time by adding 10% for hydrogen and 15% for helium.

Unfortunately, many instruments do not allow one to adjust the rate of temperature programming continuously. For these instruments, it is necessary to correct

the speed of the run to a feasible temperature programming rate by selecting a dead time corresponding to this programming rate. Even film thicknesses can be determined correctly in this way.

Composition of the test mixture

The test mixture was composed of twelve compounds in concentrations to give the same peak area (FID). The concentrations were determined by integrating peaks from eight columns with virtually no adsorption for the given compound. In order to reduce errors by remaining adsorption, 50–100 ng were injected (heavily overloading the column). Substances were of $\geqslant$95 to $\geqslant$99% purity.

Injection technique

A 1-μl of test mixture (for composition and dilution see Table IV) was injected at a splitting ratio of about 1:20 (depending on the capacity of the column).

We considered using the splitless injection technique because of the advantage of the known amount reaching the column (giving information about the capacity of the column). However, we decided against this procedure for mainly two reasons. Firstly, the large amount of solvent modifies the liquid phase temporarily (the solvent behaving as a liquid phase). This might change adsorption tests, normally in the positive sense due to solvent (or an impurity in it) blocking active sites. Secondly, we found that acids and bases, as well as the diol, are not transferred completely into the capillary owing to interaction and/or adsorption in the injector during the long residence time.

Test procedure

The procedure consists in the following steps:

(1) Cool the oven to less than 40°.

(2) Adjust the flow-rate by measuring the dead time of methane. A deviation of about 5% can be tolerated. For most instruments it is advisable to set the split flow prior to the flow measurement because changes in the split flow usually change the pressure at the column inlet.

(3) Adjust the temperature programming rate.

(4) Inject the test mixture with splitting.

(5) Immediately after injection, heat the oven to 40° and start the temperature programme.

(6) Mark on the chart the actual oven temperature once or twice at about 120–140° in order to extrapolate the elution temperature of E_{12}.

TABLE IV

COMPOSITION OF THE TEST MIXTURE

Component	*Concentration (mg/l)*	*Component*	*Concentration (mg/l)*
C_{12}-acid methyl ester (E_{12})	41.3	Nonanal (al)	40
C_{11}-acid methyl ester (E_{11})	41.9	2,3-Butanediol (D)	53
C_{10}-acid methyl ester (E_{10})	42.3	2,6-Dimethylaniline (A)	32
Decane (10)	28.3	2,6-Dimethylphenol (P)	32
Undecane (11)	28.7	Dicyclohexylamine (am)	31.3
1-Octanol (ol)	35.5	2-Ethylhexanoic acid (S)	38

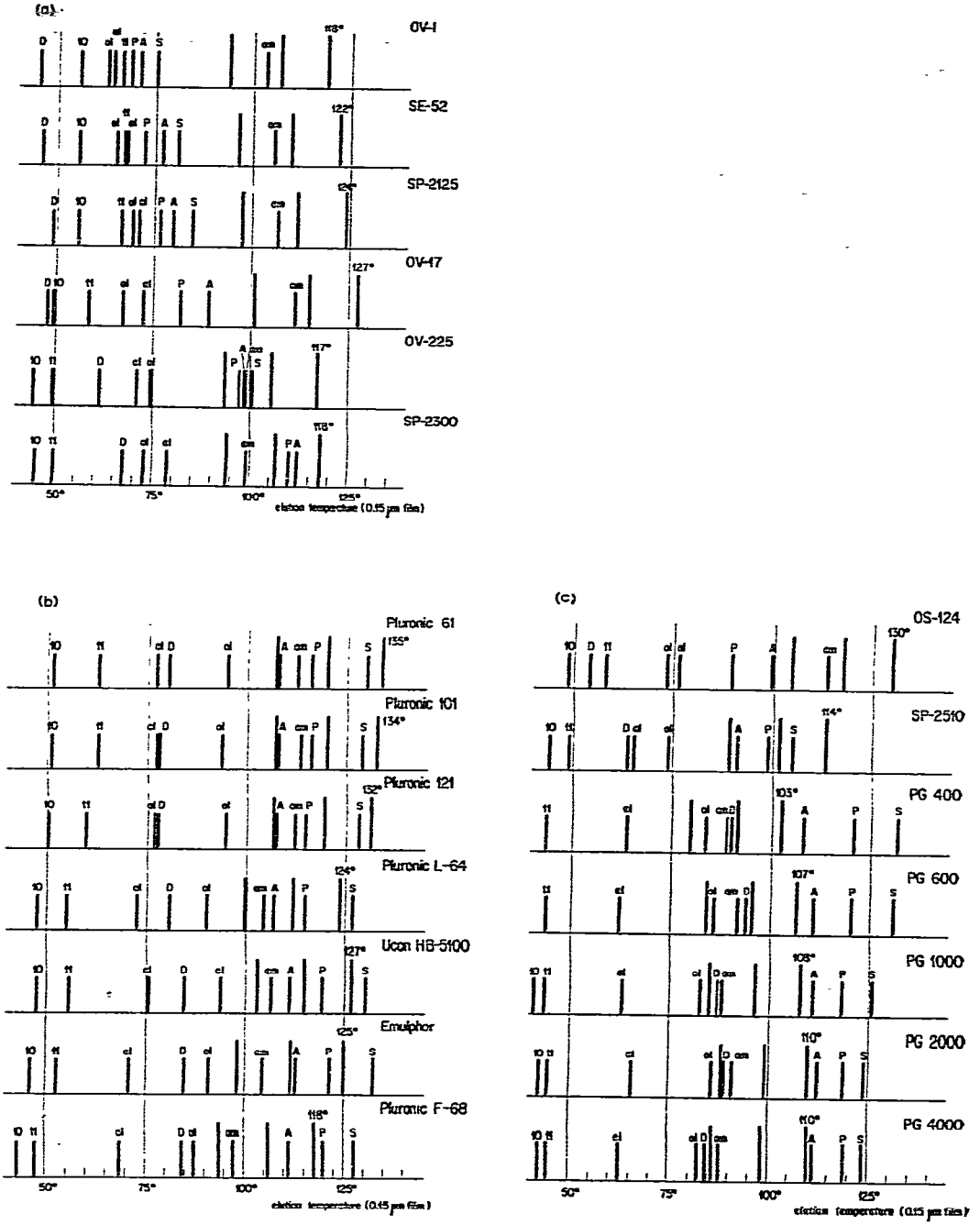

Fig. 7. Peak sequences for 20 liquid stationary phases. 10 = Decane; 11 = undecane; al = nonanal; ol = 1-octanol; D = 2,3-butanediol; A = 2,6-dimethylaniline; P = 2,6-dimethylphenol; am = dicyclohexylamine; S = 2-ethylhexanoic acid; three large peaks: methyl esters of C_{10}, C_{11} and C_{12} acids.

Peak identification

The test mixture elutes from different stationary phases with different peak sequences. Fig. 7 shows the peak sequences and the approximate elution temperatures for 20 liquid phases.

The three ester peaks are easily recognized as they are nearly equidistant (the distance between E_{11} and E_{12} is about 5% shorter). Taking into account those peaks which are expected to be 100%, we very rarely have to make further identifications.

A case which made detailed identifications necessary is illustrated by the test chromatogram (Fig. 8) of a column which was a complete failure.

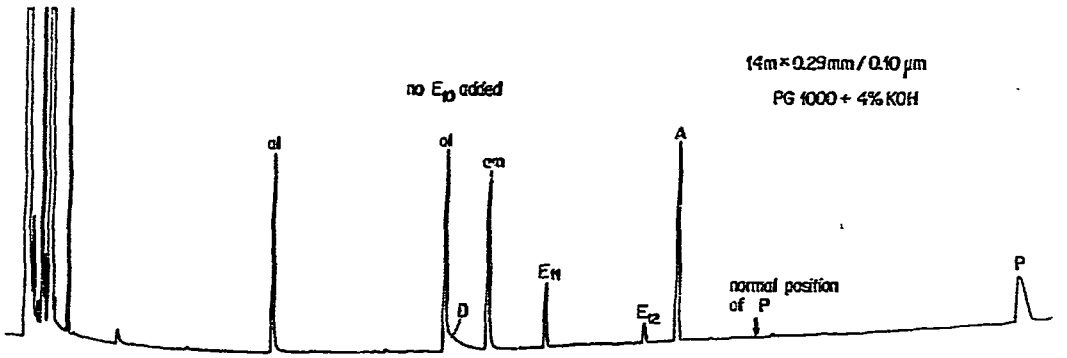

Fig. 8. Test chromatogram of a complete failure: $BaCO_3$ on soft glass, treated with KOH solution and coated by Carbowax 1000 containing 4% of KOH. The column was made in order to check if a strongly basic column elutes amines better than a column near to neutral. The test indicates that there are strongly basic active sites saponifying the methyl esters. Adsorption of alcohols is relatively high (propably caused by the same basic sites). For the same reason, the am does not elute to 100% (although secondary). P elutes with about correct area but much increased retention.

Interpretation of test chromatograms

The separation efficiency (in terms of TZ) is determined by measuring the widths at half-height ($b^{(1)}_{0.5}$; $b^{(2)}_{0.5}$) for the ester peaks using a magnifying glass fitted with millimetre scale. The measuring technique is illustrated in Fig. 9.

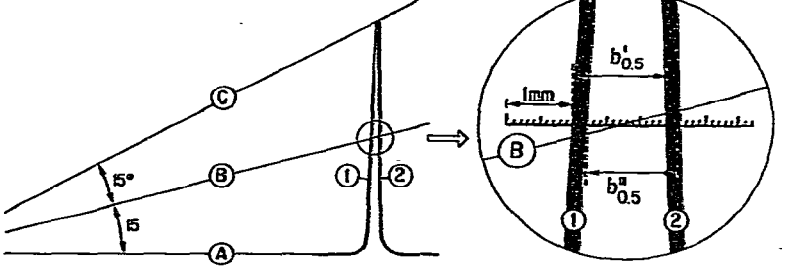

Fig. 9. Rapid and exact measurement of $b_{0.5}$ (peak width at half-height) without determination and marking half-height on the chart. Left: a straight line, perpendicular to line A and ending on line C, is cut in the middle by line B. Drawing on transparent foil or plate illuminated from the back. The chromatogram is positioned such that the baseline coincides with A, then shifted to the right or left to adjust the top of a peak on C. Right: a magnifying glass with a millimetre scale is positioned on B such that the scale crosses B in the centre between recorder traces 1 and 2; $b_{0.5}$ (1.41 mm) is the average of $b'_{0.5}$ and $b''_{0.5}$ (the thicknesses of lines 1 and 2 are often different).

TABLE V

REGISTRATION OF RESULTS FOR SOME COLUMNS MADE DURING THE DEVELOPMENT OF COLUMNS FOR AMINES AND CARBOXYLIC ACIDS

Conditioning temperature indicates the temperature to which the column was heated for one night before testing. TZ gives the separation efficiency as an average of the two ester TZ values.

No.	*Column length (m) and I.D. (mm)*	*Film thickness (μm)*	*Conditioning temperature (°C)*	*Pluronic L-64*								*Remarks*
				TS	*al*	*ol*	*D*	*A*	*P*	*am*	*S*	
P 63.1	20 × 0.30	0.15	230	33	45	100	60	95	80	80	0	$BaCO_3$ on (acidic) Pyrex
SG 103	15 × 0.29	0.11	220	30	50	100	70	100	90	100	0	$BaCO_3$ on HCl-leached soft glass, nearly neutral, inactive to amines
P 64.1	15 × 0.30	0.13	230	24	70	100	90	95	90	75	0	$BaCO_3$ on Pyrex. Low TZ due to irregular dynamic coating. Rinsed.
Recoated		0.12	230	29	65	100	90	90	90	75	0	
P 65.1	15 × 0.31	0.16	220	27	100	100	70	85	100	10	100	$BaSO_4$ on Pyrex, nearly neutral, inactive to acids
P 67.2	15 × 0.28	0.14	180	28	90	80	75	0	95	0	75	$BaCO_3$ treated with H_3PO_4, very acidic, with remaining basic sites
			210	27	75	70	40	5	95	0	60	

$$TZ = \frac{D}{b_{0.5}^{(1)} + b_{0.5}^{(2)}} - 1$$

where D is the distance between the two peak maxima.

To determine the percentage of the peak height, a line (100% line) is drawn connecting the peak maxima of the non-adsorbing peaks (alkanes, methyl esters) (see Fig. 10). For adsorbing substances, the actual peak heights are expressed as a percentage of the distance between the baseline and the 100% line.

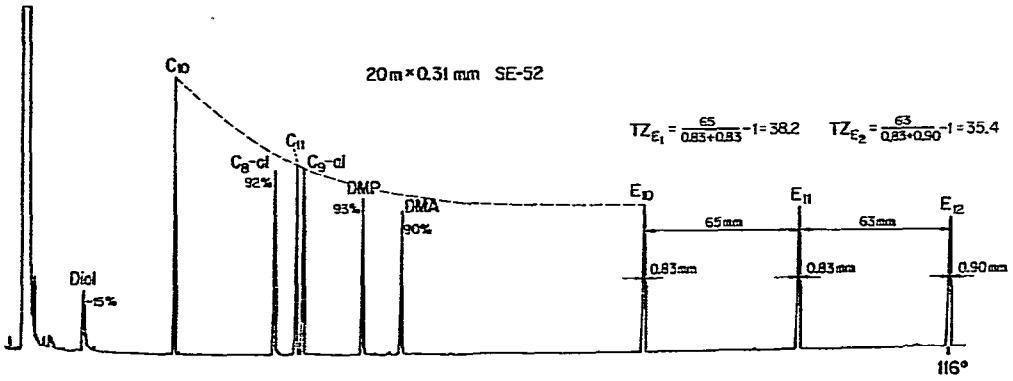

Fig. 10. Test chromatogram of a SE-52 column prepared as described elsewhere[15]: $BaCO_3$ on acid-leached Pyrex, conditioned at 240° for one night. In order to determine the percentage of the peak height of adsorbing components, draw a line connecting the peak maxima of non-adsorbing peaks (alkanes for apolar columns). The peak of the diol is poor (as is normal for non-hydroxylic phases). Adsorption by hydrogen bonding is fair for these columns. The AP test indicates a neutral support. However, the column should not be used for either phenols or anilines as quantitation is impossible. The separation efficiency is relatively high. The film thickness is 0.125 μm (static coating), confirmed by E_{12} at 116°.

The film thickness is determined, as described above, based on the elution temperature of E_{12}. Table V shows how we register our columns.

Liquid phases that cause problems

The test is based on temperature-programmed runs starting at 40°. A number of stationary phases are very viscous or solid at this temperature (*e.g.*, Carbowax 20M, FFAP and Silar 10). In some instances, this difficulty can be overcome simply by cooling the column for the shortest possible time (the stationary phase may then still behave like a liquid). For other cases, the temperature programme has to be started at 60–80°.

Other stationary phases cause problems by eluting two test components simultaneously. This cannot be avoided for complex mixtures, although by choosing the test compounds we tried to avoid coincident peaks.

In many instances, a slight change in the conditions produces a much improved separation. As the polarity of a column depends on the oven temperature,

a change in the elution temperature (or the ratio of carrier gas flow-rate to the rate of temperature programming) shifts peaks of different nature relative to each other. However, as the optimal conditions cannot then be applied, we prefer to use a test mixture without one of the affected components.

Carbowax 1000 columns very often elute the ol together with the E_{10} (depending on the film thickness). In this instance, we either use a test mixture containing no E_{10} or no ol. The mixture without E_{10} is sometimes also useful for the apolar Pluronics (61 and 121) because on these phases E_{10} may coincide with A. On SE-52 columns with a film thickness of *ca.* 0.08 μm C_{11} elutes together with al (which can be left away because aldehydes are usually very efficiently eluted from methylsilicones). The worst case we found was OV-225, which eluted A, P, am and S so close together that they influenced each other, giving no significant information. In this instance, two injections are required, one with a mixture containing A and am and another with P and S.

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