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TESTING CAPILLARY GAS CHROMATOGRAPHIC COLUMNS

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SUMMARY

A test procedure for capillary gas chromatographic columns, first published three years ago, is described in further detail, giving practical guidelines based on accumulated experience.

INTRODUCTION

Our "Comprehensive, Standardized Quality Test"[1] has been available for general use for three years, and was recently reviewed[2]. It was developed by one of us (K.G., Jr.) to meet a fundamental need of our (K.G., G.G.) laboratory, which specializes in the development of capillary gas chromatographic columns. The new test has significantly influenced our work, resulting in an obvious saving in time. Furthermore, the quantitative nature of the test has greatly improved our understanding of column quality and has facilitated new developments because of the comparability of the results. It is hard to imagine how we could have developed the persilylation procedure on the basis of our earlier testing methods. Fig. 1 gives an arbitrarily selected example of application of the test method.

While column makers obviously rely on informative and efficient testing, routine users should also apply periodic tests to their capillary columns. Only in this way can they detect alterations and be able to correlate them with their cause, *i.e.*, a given sample, the nature or amount of solvent, a given injection technique or a high temperature which the column is not able to withstand.

The three years of application have not provoked substantial modifications in the original procedure. Thus, for background information we refer the reader to the original paper[1]. For experimental purposes, however, it may be useful to provide a short description, including practical instructions, as well as some recommendations based on long-term experience.

TEST CHARACTERISTICS

Principal merits

The test yields the maximum of information about various objectives from a

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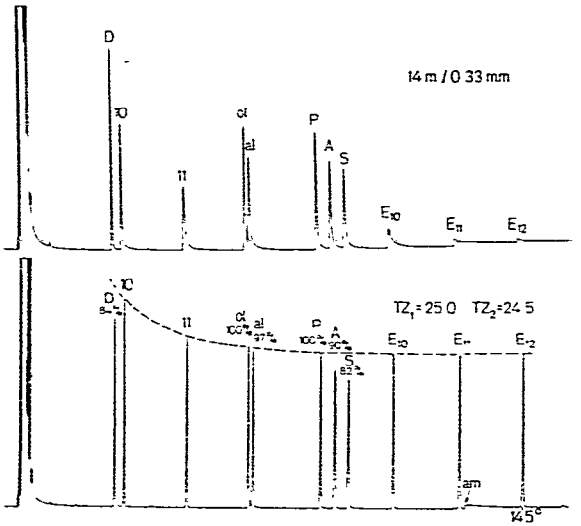

Fig. 1. Column: 14 m × 0.32 mm, coated with experimental, moderately polar silicone phase* suitable for immobilization[3]. Standardized test conditions: methane eluted at 25°C after 28 sec (hydrogen carrier); 1 μl of diluted test mixture I injected at 25°C with splitting ratio 1:30; column heated to 40°C, and temperature program (3.5°C/min) started immediately. Upper chromatogram: freshly coated column conditioned overnight at 240°C with exit connected to the flame ionization detector; flame not lit. Some bleeding was accumulated in the column exit. When the flame was lit (1.5 mm above column end!), the bleeding was carbonized. Typical effect of carbonized contamination: general adsorption, least for most polar substances (very little for D; less for ol than for al), increasing with decreasing polarity and with decreasing volatility (esters!). Lower chromatogram: test after cutting away 3 mm of column exit. Evaluation of the column: column weakly acidic (am strongly adsorbed, A 90%); inertness good (ol 100%), but not excellent (D 84%; S 82% from an acidic column); separation efficiency at maximum; relatively thick film, 0.41 μm (E_{12} eluted 20° above standard elution temperature of 125°C).

minimum of testing materials, analytical manipulation, experimental planning and time. Its chief facilities are as follows:

(1) An automatic optimization of the chromatographic conditions. Thus, no preliminary optimization run is necessary.

(2) Standardized information (from the first run) concerning four basic aspects of column quality, namely, adsorptive activity, acidity/basicity, separation efficiency and film thickness.

(3) In contrast to earlier methods which employed a large number of specific test mixtures, basically one mixture can be used regardless of the polarity of the stationary phase.

(4) Qualitative results.

(5) Full comparability of results.

In the last three years an increasing number of laboratories have utilized the test. However, an examination of the literature reveals that our test mixture is quite often used without the correct composition and without standardization, thus the results obtained are not quantitative or comparable. Typically, the test mixture used under these conditions is called a "polarity mixture". We hope that the information given here will facilitate full utilization of the method.

* Now available as OV-1701.

Temperature programming

The combined merits of this test would not be realized under isothermal conditions. Isothermal testing has to be based on experimental optimization of the conditions, which requires specific mixtures for each different quality aspect (see p. 14, point 2) and different mixtures for different stationary phase polarities. In contrast, temperature programming offers following advantages.

Automatically optimized column temperature. The optimum column temperature is dependent (under optimum flow conditions) on the molecular interactions between the sample and stationary phase and on the film thickness. While the first of these parameters may, to some extent, be theoretically predicted, the second is in many cases unknown, thus requiring repeated test runs at different column temperatures. A further problem is that a given column temperature can be optimum only for one substance within a mixture. Under programmed conditions, the individual test substances begin chromatographic migration at appropriate column temperatures, and with proper standardization, therefore, each substance will migrate automatically under optimum conditions.

Determination of film thickness. Under standard conditions and for a given stationary phase, the elution temperature of a given test substance depends only on film thickness. Consequently, film thickness can be determined in a very simple and non-destructive way.

Analysis of substances of differing volatilities. The applicability of the same test mixture to stationary phases of any polarity requires a set of test substances with a relatively broad range of volatility. Such a mixture cannot readily be analyzed isothermally.

Quantitative results. The quantitative interpretation of chromatograms is based on peak heights (since other criteria, such as peak symmetry or retention indices, are not generally applicable and are less suitable for routine use). Temperature programming facilitates this process.

Reduction of peak broadening. Temperature programming reduces the peak broadening effect due to defects in the equipment or to incorrect sampling technique, as expressed by Kaiser's[4] Q_s value. This is important in evaluation of the column rather than of the equipment or the manipulation.

It is emphasized that with temperature programming no concept of separation' efficiency other than TZ (Trennzahl, separation number) or one basically similar is applicable. An extensive discussion of TZ has appeared recently[5].

Standardization

It is evident that all the advantages of this test hold only when the test is strictly standardized. In a multi-purpose test no standardization can be applied to all functions of the test. Our standardization is set to yield optimum conditions for the determination of separation efficiency. These conditions cannot simultaneously be optimal for the determination of adsorption. The adsorption test, which has to be based on polar test substances, is more stringent for non-polar columns, since the elution temperature of polar substances decreases with decreasing column polarity, thus supporting adsorption effects.

The empirical establishment of the optimal carrier gas flow and temperature program rate was described previously[1]. However, it is often asked why the standard

conditions, in addition to a constant linear gas flow-rate, include a temperature program rate which is inversely proportional to column length. All test substances are, at first, cold trapped in the column inlet. During temperature programming they start to migrate at individual (but not readily observed) temperatures and leave the columns at individual (and easily measured) elution temperatures. The temperature range between the start and elution must be kept constant and independent of column length. A varying temperature range would result in chromatographic conditions which are not comparable on columns of different lengths, and hence in TZ measurements and adsorption/polarity determinations which are not comparable, since both depend on column temperature. If the column length is doubled at constant gas flow-rate, twice the time is required to elute a given substance within the same temperature range. This is countered by simply halving the program rate.

Our standardization produces optimal conditions for TZ only for columns with a medium range of film thicknesses (0.08–0.4 μm) and of internal widths (0.25–0.35 mm). Although TZ values found for a very wide column or for a very thick film may be slightly below the maximum value, we usually prefer to keep the same conditions for all column geometries. If such columns have to be evaluated very stringently, the test conditions are optimized empirically (*i.e.*, as they were more than 3 years ago).

Elution patterns

The order of elution of test compounds is dependent primarily on the polarity of the column. A "fingerprint" is produced which is characteristic for a stationary phase and which may be used to identify an unknown phase. Fingerprints from twenty stationary phases and elution temperatures of individual compounds were reported previously[1].

However, even under the conditions of standardized flow-rate and temperature program, these fingerprints are not absolute, being dependent on two other variables:

The published patterns are valid only for the standard film thickness (0.15 μm). A different film thickness would cause the temperature ranges of migration for all the test compounds to be shifted to lower or higher temperatures. Because of the dependence of polarity on temperature, the test compounds then "see" a column of different polarity from which they are eluted with a different fingerprint.

The published fingerprints were obtained from coatings deposited on barium carbonate surfaces (with Carbowax deactivation for apolar and medium polar coatings). Non-polar phases in particular produce substantially different fingerprints when coated on persilylated columns. These were not available when the original paper was published. Fig. 2 presents new fingerprints together with the slightly different standard elution temperatures.

Experiences with the test mixture

We have found no reason to modify the original mixture, although some of the components give less information than we had originally hoped.

The alkanes are commonly supposed not to suffer from adsorption. This is why we employed them as a basis for the "100% line". However, alkanes are often adsorbed on very inert, apolar columns. The effect is revealed by aldehyde or alcohol peaks which are higher than those of the alkanes. Quantification of adsorption is, of course, not possible in such cases.

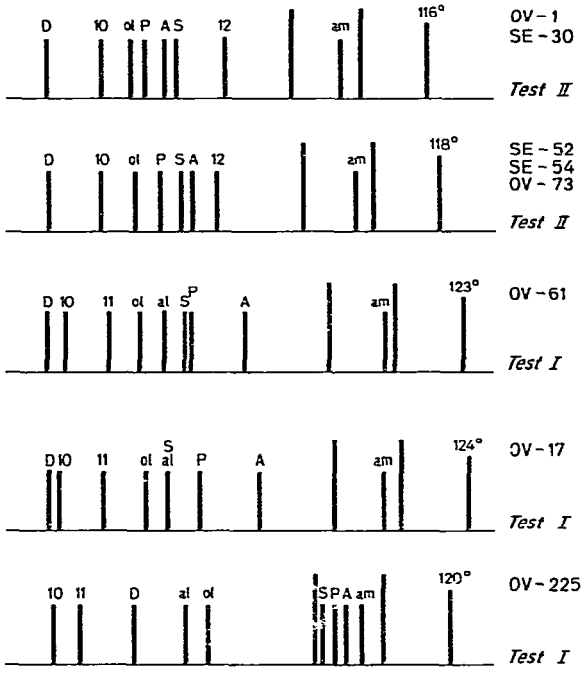

Fig. 2. Elution patterns from persilylated columns. Note the different test mixtures used for different polarities. The unlabelled tall bars represent the esters E_{10}–E_{12}. The given peak sequences and the elution temperatures for E_{12} are correct for the arbitrarily selected standard film thickness of 0.15 μm.

The esters have a dual purpose in this test; they permit determination of TZ, and they serve as a second standard for 100% peak height. Although adsorption of esters is more likely than of alkanes, on columns of reasonable quality it is negligible. It is therefore our policy not to attempt quantification of adsorption on poor columns on which esters are adsorbed.

2-Ethylhexanoic acid is a strongly acidic component which causes problems on apolar columns. A free carboxylic acid is clearly incompatible with an apolar liquid phase; when even less than 1 ng of 2-ethylhexanoic acid has entered the column, the column is already overloaded as can be observed from distorted (leading) peaks. It was this problem which led us to select a branched chain acid. However, there was no appreciable improvement with 2,2-diethylbutyric acid, which has even greater steric hindrance of the carboxyl group. Lacking a strongly acidic and simultaneously sufficiently lipophilic test substance of suitable volatility, we are resigned to interpreting the elution of the free acid from apolar columns on the basis of peak area instead of peak height.

PRACTICAL GUIDELINES

The test mixture

The test mixture is crucial and is the only somewhat demanding part of the method. We have learned that the lack of a suitable mixture or of one of appropriate composition is the major limitation of the test. Therefore, high priority has to be given to this point.

Several test substances, especially acidic and basic ones, are commercially available in various purities and may undergo alteration depending on the storage conditions. A specific problem is 2,3-butanediol which is often available as a mixture of isomers. To circumvent these difficulties, we have encouraged Fluka (Buchs, Switzerland) to distribute the following two test mixtures:

No. 86499, test mixture I (general mixture)

No. 86501, test mixture II (modified mixture for non-polar phases; see under "modified test mixtures")

We have carefully checked the compositions of both mixtures and will repeat the check periodically for this and each new batch. The regular package contains 5 ml of concentrated mixture. A 1-ml volume of the mixture dissolved in 20 ml pure hexane (without volumetric precision) yields a dilute solution ready for use. The durability of the diluted mixture depends on the storage and handling conditions and may vary between 1 month and 2 years. The concentrated mixture has unlimited durability when stored in a freezer. It is supplied with a sheet giving brief information on standardization, and on the interpretation of test chromatograms.

For those who prefer to prepare the test mixture themselves, the individual components and their proportions are given in Table I. If other than Fluka products are used, care must be taken to ensure that only one isomer of butanediol is present. The two alkanes are combined in one 20-ml vial and the three esters in another (nine vials for the twelve substances). To each vial, 20.0 ml hexane are added, except for the vial containing butanediol, the solvent for which is chloroform. After use, the solutions are kept in a freezer. They can be stored for years. The concentrated test mixture is prepared by transferring 1.00 ml of each of the above solutions into a 10-ml vial. The resulting mixture should be stored in a refrigerator. The dilute test mixture is

TABLE I

AMOUNTS OF SUBSTANCES USED FOR THE PREPARATION OF THE CONCENTRATED TEST MIXTURE

Fluka catalogue No.	*Substance*	*Code*	*Amount dissolved in 20.0 ml solvent (mg)*
21479	Methyl decanoate	E_{10}	242
94120	Methyl undecanoate	E_{11}	236
61689	Methyl dodecanoate	E_{12}	230
30560	*n*-Decane	10	172
94000	*n*-Undecane	11	174
44010	*n*-Dodecane*	12	176
74850	1-Octanol	ol	222
76310	Nonanal	al	250
18965	2,3-Butanediol	D	380
39520	2,6-Dimethylaniline	A	205
41350	2,6-Dimethylphenol	P	194
36520	Dicyclohexylamine	am	204
03300	2-Ethylhexanoic acid	S	242

* Only for test mixture II.

prepared by transferring 1 ml of the concentrated mixture to a 20-ml vial and making up to 20 ml with hexane. Fresh solution is made from the concentrated mixture whenever a yellow colour appears in the dilute solution or when any doubt arises as to its composition.

Solutions for identification

Unknown or mixed phases, as well as columns with extreme geometries, may produce fingerprints which lead to doubtful peak identity. In such cases, co-chromatography of the test mixture with solutions of the individual substances should provide the missing information. For this purpose, small amounts of standard solutions (primarily of ol, S, am, A or P, see Table I) diluted 1:50 may be employed.

Modified test mixtures

Our test mixture is designed to give a minimum of peak coincidences on all phases. Incidental overlappings cannot be avoided, however. When a column producing overlapping peaks is of special interest, it may be worthwhile to prepare a specific concentrated test mixture for it.

An example is a modified mixture for non-polar columns, on which a coincidence of *n*-undecane, 2,6-dimethylphenol and nonanal is likely to occur. We omit nonanal, since non-polar, inert columns tend not to adsorb aldehydes, and we replace *n*-undecane by *n*-dodecane, which has no close neighbours on non-polar columns.

Procedure

(1) Cool the oven to room temperature or at least to less than 40°C. Efficient cooling is particularly important with thin films.

(2) Set a suitable carrier gas pressure, and adjust the splitting ratio. Inject a few microlitres of methane (most fuel gases contain enough methane), and measure with a stop-watch the time between injection and the first recorder signal. Adjust the time to the standard time (±5%) of 2 sec/m for hydrogen, 3.5 sec/m for helium. Changing the split flow now may significantly affect the carrier gas pressure!

(3) Set the temperature program as obtained by inter- or extrapolation from the following basic data: column length 10 m, 5.0°C/min with hydrogen, 2.5°C/min with helium; column length 50 m, 1.0°C/min with hydrogen, 0.5°C/min with helium.

(4) Inject the test mixture under conditions that allow *ca.* 2 ng of a single test substance to enter the column (*e.g.*, 1 μl with splitting ratio of 1:20 to 1:50, depending on injector design).

(5) Immediately after the injection, heat the oven to 40°C (for very thin films, to 30°C) and start the temperature programming.

(6) Within the temperature range in which the third ester is eluted (on most columns, 110–140°C), make two marks on the recorder chart noting the actual oven temperature.

(7) At the end of the run, inter- or extrapolate the elution temperature of the third ester.

(8) Draw the "100% line" over the two alkanes and the three esters as shown in Fig. 1.

(9) Express the height of the remaining peaks as a percentage of the distance between the baseline and the 100% line.

(10) Determine TZ as an average of TZ E_{10}/E_{11} and TZ E_{11}/E_{12}.
(11) Determine the film thickness using the nomogram[1].

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