Performance of Capillary Columns for High-Temperature Gas Chromatography

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Abstract

The developments in stationary-phase synthesis and capillary column technology have opened new perspectives in the analysis of high-molecular-weight compounds (600 daltons) and thermolabile organic compounds by high-temperature-high-resolution gas chromatography. This branch of high-resolution gas chromatography deals with analysis performed up to 390°C oven temperature (with some applications going up to 420°C and even a few applications to 450°C maximum). The technique has been applied to many different fields of science (e.g., organic geochemistry, environmental chemistry, archeology, and natural product research). Apolar and medium-polar gum phases can now be operated at temperatures from 400 to 480°C, but these higher temperatures are seldom used because of the thermostability of the material used to make the capillary tubing. This paper shows the performance of nine commercial high-temperature columns when used in routine applications.

Introduction

Historical

In 1957, Marcel Golay presented the first theoretical treatment of capillary columns (1). Since this report, the main objective of chromatographers has been the development of more inert and thermostabile capillary columns. The first capillaries employed in the developmental stage of the technique were manufactured from polymeric materials (Tygon, Teflon, and nylon) and metals (aluminum, nickel, copper, stainless steel, and gold). Polymeric materials have obvious temperature limitations, whereas metallic materials had the disadvantage of catalytic activity and lack of compatibility with the stationary phase. Glassy materials were clearly superior to those obtained with the others (2,3). Attention was then focused on glass capillary columns, which were brittle and surface active. Although, glass-surface modifications followed by persilylation (3) resulted in highly inert and stationary-phase compatible capillaries.

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The insufficient thermal stability of the stationary phases that were commercially available (associated with the difficulty in producing chemically inert and thermally resistant capillary tubes) slowed the development of high-resolution gas chromatography (HRGC) and hindered the establishment of high-temperature (HT)-HRGC. These limitations were overcome by the work of Grob in the late 1970s and 1980s (3), followed by his work together with Blum in establishing the basis for the development of HT-glass capillary columns (4).

There are a reasonable number of reports about the use of HT-HRGC as a standard technique in many gas chromatographic (GC) laboratories (5). The term usually denotes temperature-programmable GC operation with final column temperatures of 370°C or higher. Today, the apolar and medium-polar high-temperature capillary columns can be conveniently operated at temperatures up to 420°C (5).

A milestone in the development of HRGC was the introduction of the fused-silica capillary column by Dandenau and Zerenner in 1979 (6). This new material (because of its better mechanical resistance and high flexibility) is responsible for the widespread use of the technique, and it has greatly extended the range of GC applications. Therefore, less-skilled technicians and outsiders from the GC field could easily apply the technique. However, for HT-HRGC, there are limitations related to the use of the "common" silica capillary columns such as the low-temperature stability (320°C temperature limit, in some cases extending to 325–350°C) of the material used in the coating of the capillary column. Another approach was the development of the aluminum-clad fused-silica capillary columns, which are capable of withstanding temperatures up to 500°C (7,8). There are reports in literature that aluminum-clad columns are difficult to disconnect from the oven after 24 h of use at 400°C (9). The main advantage of the commercial fused-silica capillary columns is their flexibility when compared with glass capillaries; however, there are limitations to the use of the polyimide and aluminum coatings. Aluminum-clad fused-silica capillary tubing suffers from differential expansion coefficients between the aluminum layer and silica. Polyimides (even high-temperature polyimides) are progressively degraded at high temperatures. In certain products, the adhesion

of the polyimide coating can easily be compromised at high temperatures, resulting in a peeling off of the coating. On the exposed fused-silica surface, microfissures can form and expand rapidly into cracks, leading to spontaneous breakage of the flexible capillary coil (5,10,11).

Today, fused-silica capillaries coated with polyimide are used for most commercial columns. For the use of fused-silica capillary columns at high temperatures, new polyimides for high-temperature applications have been developed. This second generation of polyimide coating is said by the manufacturers to withstand heating up to 400°C without thermal decomposition of the polyimide (5,10), but the use of this type of capillary column in high-temperature applications (mainly higher than 400°C) has been criticized by several members of the scientific community. There are reports that the limit of use of these capillary columns is between 370°C and 400°C (3,7–9,11), whereas other reports state that this kind of coating is stable up to 450°C without serious loss in flexibility (12).

In this study, we evaluated commercial capillary columns (aluminum-clad and polyimide-coated fused-silica) for their thermal stability, activity, and dependence on column temperature.

Previous results with usual HRGC columns

In 1979, Grob, Jr. (13) discussed the possibility of triglyceride analyses using glass capillary columns coated with SE-52 (100% methyl-silicone, 10-m × 0.3-mm i.d., 0.12-µm film thickness) and observed that after approximately 20 runs at up to 370°C containing triglycerides of ripe seed oil (with a high content of erucic acid and ranging in molecular weight to above 1000 daltons), column properties did not change noticeably. He showed that prolonged periods of capillary column conditioning does not promote extensive stationary-phase loss and destruction of the capillary column, which was a widespread misconception. Capillary columns coated with a standard stationary-phase SE-54 (1% vinyl/5% phenyl/94% methyl-silicone, made in our laboratory according to reference 3 and having a maximum temperature between 300°C and 325°C) were subjected to similar experiments as realized in this study (14). A period of 150 h at 350°C promoted only the loss of approximately 10% of the film thickness in all capillary columns. Also observed was a decrease in the separation efficiency and an increase in basicity and adsorptive activity after 12 h at 350°C. In several columns, initial inertness was partially restored by rinsing the column with two volumes of hexane and two volumes of dichloromethane (Figure 1). This showed that prolonged conditioning at high temperatures did not irreversibly affect the capillary columns and confirmed Grob, Jr.'s data that capillary columns coated with standard stationary phases (100% methyl-silicone and 5% phenyl/95% methyl silicone) could be used at up to 350–370°C without significant degradation.

A significant fact was observed when commercial fused-silica capillary columns (HP-1 and HP-5, Hewlett-Packard) and SE-54 glass Duran-50 capillary columns (made in our laboratory) were submitted to treatment at 350°C for 8 h without a carrier gas. The column was installed in a chromatograph, the system was pressurized for several minutes and conditioned, and the carrier gas inlet valve was shut before heating. All columns (total number of six: three commercial fused-silica capillary columns and three glass Duran-50 capillary columns) showed the same behavior, most notably the decrease in acidity and the total adsorption of the three esters (Figure 2). This behavior is compatible with basic columns. A similar result was presented by Grob, Jr. et al. (15) with a column of soft glass (deactivated with $BaCO_3$) treated with a KOH solution and coated with Carbowax 1000 containing 4% KOH. All columns submitted to this heating process developed a strong basicity and were able to elute amines better than the best capillary column to date (including commercial capillary columns). However, they did not elute the ester peaks, because (as suggested by Grob, Jr. et al.) strong basic sites were saponifying the methyl esters and the acids formed were strongly adsorbed.

Another development in the technology of the capillary column production was the application of factorial design in order to develop fused-silica capillary columns using mathematical models, which resulted in the production of capillary columns of superior performance with significant improvements in reproducibility and thermostability at up to 350°C (16). Therefore, a second generation of high-resolution capillary columns (the "low bleed" columns) was developed. These columns used special surface deactivation and siloxane chemistry, which enhance the chromatographic performance of siloxane polymers. They showed increased sensitivity because of a decrease in the signal-to-noise ratio (17).

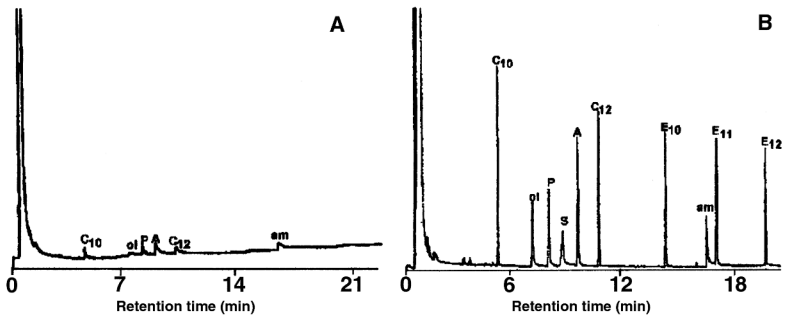

Figure 1. Grob tests of the glass Duran-50 capillary column (SE-54, 20 m × 0.30 mm × 0.30 µm) after thermal treatment at 350°C for 150 h (A) and after rinsing the column with two volumes of hexane and two volumes of dichloromethane (B): decane, C_{10}; dodecane, C_1; methyl decanoate, E_{10}; methyl undecanoate, E_{11}; methyl dodecanoate, E_{12}; 1-octanol, ol; 2,6-dimethylphenol, P; 2-ethylhexanoic acid, S; 2,6-dimethylaniline, A; dicyclohexylamine, am.

Column performance evaluation

Polar, basic, and acidic compounds are adsorbed on active surfaces such as inner capillary walls. In GC, the ideal interaction of solutes occurs only with the stationary-phase coating. Despite modern column deactivation technology, small amounts of polar solutes may be strongly adsorbed on surface-active sites. Therefore, different groups that have been involved in the development of capillary columns have proposed different mixtures for column performance testing (e.g., inertness and resolution).

The performance of a capillary column can be evaluated with a test mixture in which components and resulting peak shapes serve as monitors of column efficiency and diagnostic probes for

adverse adsorptive effects and the acid–base character of the column. One of the most commonly applied test mixtures is the one developed by Grob et al. (15,18). This "Grob test" aims the general evaluation of the capillary column (i.e., the separation efficiency, polarity, and adsorptive activity) in the low temperature range (up to 150°C).

For column evaluation at T > 150°C, other test samples are necessary that are mainly intended to evaluate the catalytic activity and adsorptive effects of the column, which are known to increase with working temperature. Several test mixtures were proposed for temperatures above 150°C: the Donike test (19) for temperatures up to 300°C and both the Triton test (20) and triacylglycerol mixture (21) for temperatures above 300°C.

According to Grob et al. (15), an ideal capillary column should be well-deactivated, have excellent thermal stability, and high-separation efficiency. In the Grob test, the first procedure measures peak heights as a percentage of that expected for complete and undistorted elution. The technique encompasses all types of peak deformations (broadening, tailing, and irreversible adsorption). Adsorption may cause: (*a*) broadened peaks of Gaussian shapes, (*b*) a tailing peak with more or less the correct peak area, (*c*) a reasonably shaped peak with reduced area, and (*d*) a skewed peak of correct area having an increased retention time.

The Grob test provides a semiquantitative description of the activity expressed as a percentage of the peak height in relation to a line drawn by connecting the peak maxima of the nonadsorbing peaks (such as alkanes and methyl esters) present in this test mixture. The Donike test also provides a semiquantitative description, but by the quotient:

$$Q = (A_{fa\text{-}TMS}) / A_{alk} \qquad \text{Eq. 1}$$

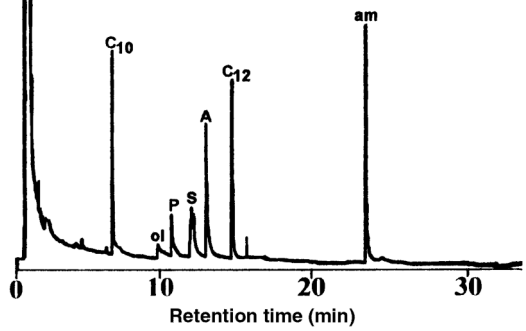

Figure 2. Grob test of a capillary column (HP-5, 25 m × 0.25 mm × 0.25 µm) after thermal treatment at 350°C for 8 h without carrier gas: decane, C_{10}; dodecane, C_{12}; methyl decanoate, E_{10}; methyl undecanoate, E_{11}; methyl dodecanoate, E_{12}; 1-octanol, ol; 2,6-dimethylphenol, P; 2-ethylhexanoic acid, S; 2,6-dimethylaniline, A; dicyclohexylamine, am.

where Q is the activity factor, $A_{fa\text{-}TMS}$ is the peak area of the fatty acid-TMS ester, and A_{alk} is the peak area of the corresponding *n*-alkane. All values of Q that are less than 1 imply activity.

The Donike test monitors catalytic activity up to 300°C. The test has not been extended for higher temperatures, because fatty acids and alkanes with more than 36 carbon atoms are not widely available and their solubility in organic solvents is very poor.

For the evaluation of a column's catalytic properties above 300°C, the Triton test can be used (20). The original Triton test contains methyl- and TMSs of the detergent, Triton-X100, or octylphenol-poly(ethylene glycol) ether, but for stationary phases with 50% phenyl or more, the doublets of higher oligomers overlap. Base-line separation can be achieved by replacing the methylethers with the corresponding ethylethers (22). The peaks of the oligomers form a distribution curve, and the heaviest oligomers elute at approximately 420°C. This test was the first simple test proposed for the rough evaluation of the catalytic activity of capillary columns at temperatures above 300°C, but its main disadvantage is that it is not very sensitive to catalytic and thermal degradation because the silyl ethers are almost as stable as the alkyl ethers (21).

Another proposed test for capillary columns is the analyses of a triacylglycerol mixture, which gives information about the separation power, catalytic activity, and bleeding of the capillary columns at temperatures between 300°C and 420°C. The elution order of the saturated and unsaturated triacylglycerols also gives information about the selectivity of the stationary phase (21).

Table I. Nomenclature of the Triacylglycerols Used as a Test Mixture

	Triacylglycerol	ΔC*	Abbreviation	Mole mass Fatty acid	Mole mass (daltons)
1	Triacetin	6	AcAcAc	3xC2:0	218.0
2	Tributirin	6	BBB	3xC4:0	302.2
3	Tricaproin	6	CCC	3xC6:0	386.4
4	Tricaprilin	6	ClClCl	3xC8:0	470.6
5	Tricaprin	6	CiCiCi	3xC10:0	554.8
6	Trilaurin	2	LaLaLa	3xC12:0	638.9
7	1,2-Dilauroyl-3-myristoyl	2	LaLaM	2xC12:0, 1xC14:0	667.0
8	1,2-Dimyristoil-3-lauroyl	2	MMLa	1xC12:0, 2xC14:0	695.1
9	Trimyristin	2	MMM	3xC14:0	723.1
10	1,2-Dimyristoil-3-palmitoyl	2	MMP	2xC14:0, 1xC16:0	751.2
11	1,2-Dipalmitoyl-3-myristoyl	2	PPM	1xC14:0, 2xC16:0	779.2
12	Tripalmitin	2	PPP	3xC16:0	807.3
13	1,2-Distearoyl-3-myristoyl	2	SSM	1xC14:0, 2xC18:0	835.4
14	1,2-Distearoyl-3-palmitoyl	2	SSP	1xC16:0, 2xC18:0	863.4
15	Tristearin	6	SSS	3xC18:0	891.5
16	Triarachidin	6	AAA	3xC20:0	957.7
17	Tribehenin	–	BeBeBe	3xC22:0	1059.8

* The difference in number of carbons between consecutive eluting peaks used to normalize the TZ values calculated for each pair. Normalized TZ values = TZ / (ØC × column length).

We decided to use the Grob test as a reference test for high-temperature capillary column evaluation despite the fact that its temperature limit is approximately 150°C. Our objective in this study was to evaluate the thermal stress suffered by these columns when heated for prolonged periods at high temperatures, which should decrease inertness and film thickness (4). Therefore, the actual probing of the behavior at high temperature is not mandatory. Conversely, there is a large amount of information in literature concerning the Grob test applied to several different stationary phases (including high-temperature stationary phases) and different types of columns. Also, it has the widest range of active site probes and is the only one that incorporates the evaluation of film thickness. The elution temperature of the E_{12} (methyldodecanoate) obtained by the Grob test can be used to determine film thickness. Grob et al. (15,18) demonstrated that there is a logarithmic dependence of the elution temperature on the film thickness and established an experimental nomogram in which the experimental film thickness can be determined. Even though the extension of its application to any column type can be questioned, the relative comparison of E_{12} elution temperatures (before and after heating the column for prolonged periods) is a good indicator of film-thickness changes.

High-temperature resolution was measured by the use of the triglycerides test through the determination of Trennzahl (TZ) (the separation number) of consecutive homologues.

Experimental

For the evaluation of the resolution of the high-temperature capillary columns at temperatures above 150°C, a triglycerides mixture was used (Table I).

The seventeen triglycerides were used in equal concentrations (w/v). To compare the resolution of the column for different pairs of the triglyceride peaks, it was necessary to normalize TZ. Therefore, we decided to divide the value of TZ by the difference of the number of carbons (resulting from the differences of the triglyceride chains, see Table I) These normalized values were used to evaluate the capillary columns at high temperatures.

Columns

All columns used in this evaluation are in Table II. They were donated upon written request from more than 50 dealers and manufacturers of capillary columns (23). The letter requesting columns stated that they were to be used in the evaluation of column performance and applied work. There are no indications that the columns were taylor-made for this donation or were over-the-counter samples.

Standards

All triacylglycerols were obtained from Sigma (St. Louis, MO) The abbreviations used for the triacylglycerols are given in Table I.

A Grob test mixture was prepared in-house from pure standards according to Grob, Jr. et al. (15).

Chromatographic conditions

An on-column injector (Carlo Erba, Rodano, Italy) was mounted on a Hewlett-Packard (Palo Alto, CA) Model 5890-II GC. Hydrogen was used as the carrier gas at a linear velocity of 50 cm/s (40°C). GC data were acquired and processed with an HP 3396-II integrator. The flame ionization detector (FID) and the on-column injector were operated at 400°C and room temperature, respectively. The sample volume injected was 0.2 µL.

Analysis conditions

For the Grob test, the column temperature was maintained at 40°C for 0.5 min and then programmed at 50°C per length of the column per minute to 150°C. For the triglycerides, the column temperature was maintained at 40°C for 0.5 min and then programmed at 20°C/min to 400°C and held for 20 min.

Results and Discussion

Preliminary column performance test

Different manufacturers use different mixtures or samples for the quality control of their columns. For example, BGB analytik (Rothenfluh, Switzerland) uses the Grob test; Quadrex (Woodbridge, CT) uses Canadian wax and triglycerides for butter; J&W Scientific (Folsom, CA) and SGE (Austin, TX) use the "Hindelang" Round-Robin test; and Chrompack (Middelburg, The Netherlands) uses a mixture of C_{14}, C_{15}, C_{16}, C_{18}, and C_{20} *n*-alkanes). Therefore, it is not possible to compare their factory-evaluated characteristics. We decided to run Grob tests for all columns (Table III). The analyses of the initial Grob test results

Table II. High-Temperature Capillary Columns Evaluated

Column	Code	Stationary phase	Characteristics	Manufacturer
1	BGB-Silaren #52582	30% Diphenyl/40% sildiphenylene-ether/30% dimethyl-silicone	Polyimide-coated (15 m, 0.25-mm i.d., 0.12-µm d.f.)	BGB Analytik
2	Al-Clad Series #71014H	5% Phenyl/95% methyl-silicone	Aluminium-clad (15 m, 0.25-mm i.d., 0.12-µm d.f.)	Quadrex
3	007-65HT #80701G	65% Phenyl/35% methyl-silicone	Polyimide-coated (15 m, 0.25-mm i.d., 0.12-µm d.f.)	Quadrex
4	CP-SimDist #261063	100% methyl-silicone	Metal-clad (10 m, 0.53-mm i.d., 0.10-µm d.f.)	Chrompack
5	HT-5 #274327	5% Phenyl/95% methyl-silicone	Aluminium-clad (12 m, 0.22-mm i.d., 0.10-µm d.f.)	SGE
6	HT-5 #1319B01	5% Phenyl/95% methyl-silicone	Polyimide-coated (12 m, 0.22-mm i.d., 0.10-µm d.f.)	SGE
7	DB1-HT #8722821A	100% methyl-silicone	Polyimide-coated (15 m, 0.25-mm i.d., 0.10-µm d.f.)	J&W Scientific
8	DB5-HT #8722471B	5% Phenyl/95% methyl-silicone	Polyimide-coated (15 m, 0.25-mm i.d., 0.10-µm d.f.)	J&W Scientific
9	DBHT-SIMD #8804712F	100% methyl-silicone	Metal-clad (5 m, 0.546-mm i.d., 0.15-µm d.f.)	J&W Scientific

showed that the columns used in this study have high inertial and resolution power—only the "megabore" capillary columns (columns 4 and 9 of Table III) showed adsorption because of acid–base effects.

The traditional inert capillary columns commonly show a minute residual activity attributed to the silanol groups on the support surface that manage to survive the persilylation and analogous strong deactivating treatments. In the high-temperature, high-resolution capillary columns, the residual silanol groups are largely eliminated by the condensation process with the stationary phase (4). This fact increases the inertness, which withstands several days of heating beyond 300°C. Preliminary quantitative general information and catalytic activity of the capillary columns were obtained by running a sequence of the comprehensive test (Grob test) intercalated with 12-h conditioning periods with increasing final temperatures (250°C, 300°C, 325°C, 350°C, 370°C, and 390°C) (see Figure 3).

After being conditioned at 370°C, all columns in Figure 3 showed increased activity and decreased column efficiency. Figure 4 shows the Grob tests of column 1 (BGB-silaren) before the previously mentioned heating/testing sequence and after the treatment at 390°C. After the 390°C treatment, all columns used in this study showed adsorption problems, because all peaks (including the *n*-alkanes) had tailing. These phenomena caused by yet-unknown mechanisms result in all types of adsorption as measured by the Grob test (i.e., hydrogen bonding and acid–base effects).

The loss of approximately 20% of the film thickness in all capillary columns as determined by the Grob test procedure (15,18) was observed after conditioning the columns at 390°C for 12 h.

Column performance after high-temperature use

According to the Grob test analysis, after being conditioned at 370°C, all columns showed increased activity and decreased column efficiency (see Figure 1). In order to investigate how this affects the resolution at high-temperature conditions, the triglyceride mixture was injected in triplicate. The resolution of all capillary columns was similar (Figure 5) and the results were considered satisfactory (Figure 6). The TZ for the pairs of triglycerides between 1,2-dilauroyl-3-myrostoyl-glycerol and tristearin-glycerol (MW between 667 and 891.5 daltons) gave TZ values near 8. However, in columns 1 and 6, more activity was shown and the tribehenin-glycerol did not elute (Figure 5).

Sample capacity increases as the column diameter increases.

Table III. Initial Inertness and Resolution of the Capillary Columns According to the Grob Test

Column*	ol	P	S	A	am	TZ
1	98	98	8	100	62	26.8
2	92	95	5	98	65	27.0
3	92	93	3	99	60	26.8
4	100	100	nd‡	89	nd	8.0
5	100	90	4	95	75	21.8
6	96	95	5	94	80	21.6
7	96	96	7	93	85	26.8
8	98	99	8	90	80	25.5
9	100	96	nd	90	80	4.0

* Column numbers correspond with those of Table I.
† Percentage in relation to the "100% line" drawn from the *n*-alkanes and methyl ester peaks: 1-octanol, ol; 2,6-dimethylphenol, P; 2-ethylhexanoic acid, S; 2,6-dimethylaniline, A; dicyclohexylamine, am; and Trennzahl, TZ.
‡ nd, Peak not detected.

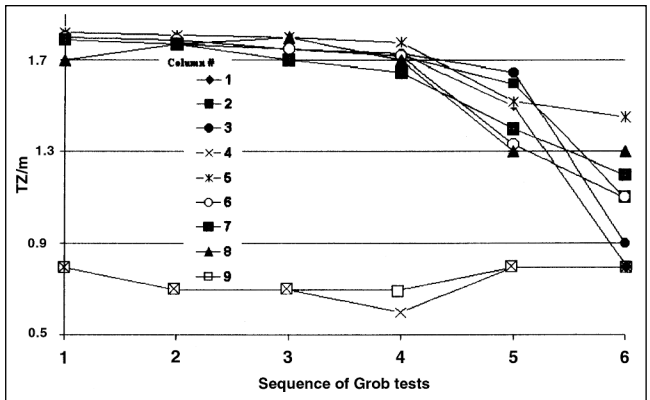

Figure 3. Effect of thermal treatment over TZ (taken per meter) as determined by the Grob test performed after each heating period.

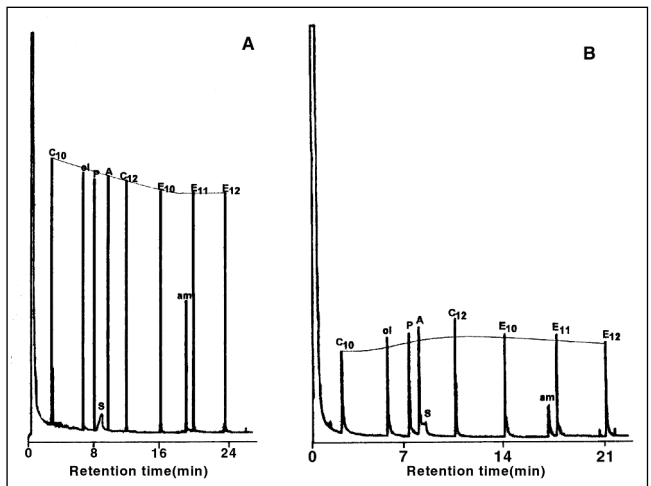

Figure 4. Grob tests of column 1 (BGB-Silaren) before starting the sequence of heating/Grob test (A) and after thermal treatment at 390°C (B): decane, C_{10}; dodecane, C_{12}; methyl decanoate, E_{10}; methyl undecanoate, E_{11}; methyl dodecanoate, E_{12}; 1-octanol, ol; 2,6-dimethylphenol, P; 2-ethylhexanoic acid, S; 2,6-dimethylaniline, A; dicyclohexylamine, am.

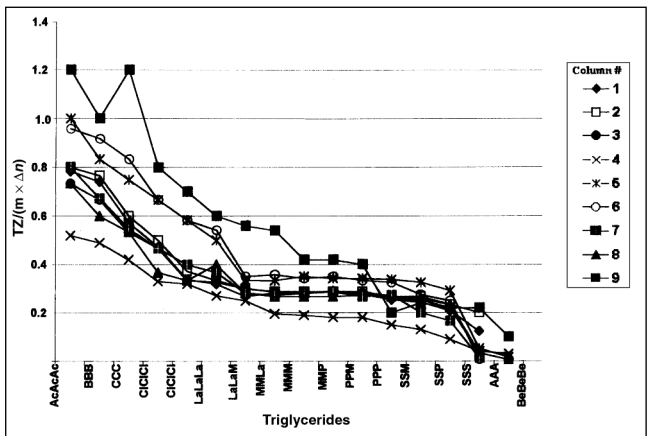

Figure 5. Resolution of the triglycerides in all capillary columns evaluated determined by TZ (taken per meter times the difference of the CH_2 number, *n*).

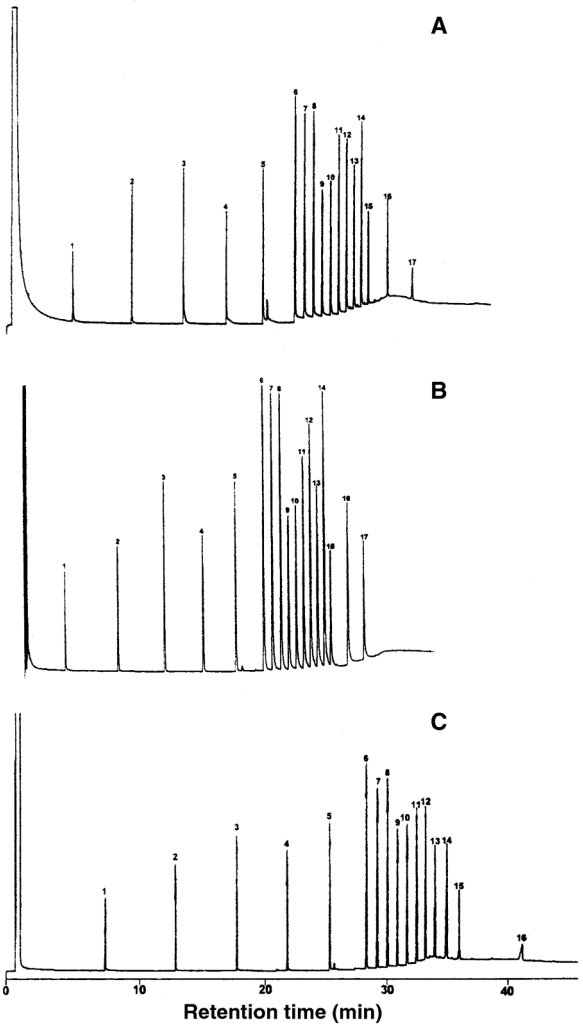

Figure 6. Representative chromatograms of the triglyceride analyses: column 5, A; column 4, B; and column 3, C. (column specifications found in Table II).

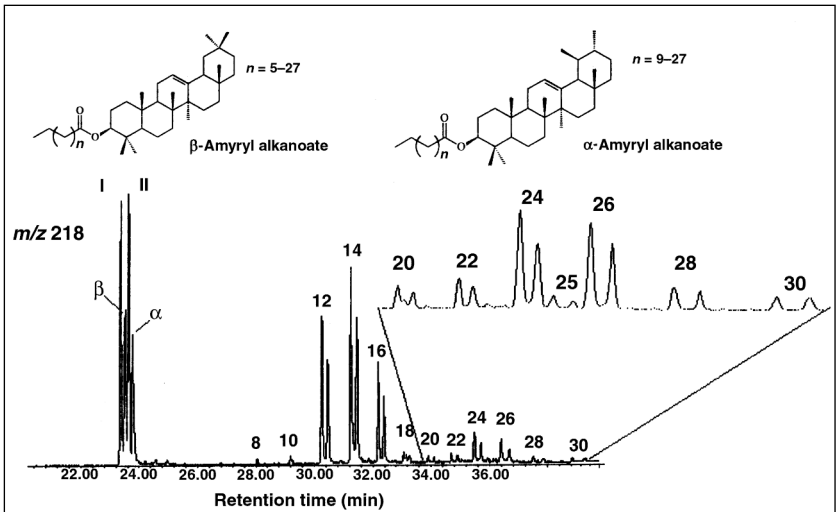

Figure 7. HT-HRGC of a dichloromethane crude extract of *Croton hemiargyreus* using column 8 programmed from 40°C (0.5 min) at 10°C/min to 390°C and then held for 20 min. The FID and on-column injector were operated at 400°C and room temperature, respectively. Triterpene not characterized, I; 3-keto-urs-12-ene, II; α-amyrin, α; and β-amyrin, β. Numbers above the peaks represent the number of carbons in the fatty acid chain (n + 3). For each pair, the β-amyryl derivative elutes first.

The selection of a column's internal diameter may be based on the type of sample inlet system that is being used. For very simple mixtures, the megabore columns with short lengths (≤ 10 m) have similar results for the resolution of the higher molecular-weight triglycerides, triarachidin, and tribehenin when compared with the other capillary columns.

As expected, the intrinsic resolution per meter (Figure 3) (measured by TZ/m) showed that the wide-bore capillaries could not be considered as high-resolution capillary columns. Their separation efficiency was much lower than that of typical capillary columns (0.25-mm i.d.). However, after intense thermal stress, their poor performance remained the same, compared with some of the high-resolution capillary columns loosing their high-resolution characteristics (i.e., columns 1, 2, 3, 6, and 7 in Figure 3).

Bleeding of the high-temperature capillary columns

Usually, decomposition (bleeding) products from the stationary phase or impurities from the carrier gas are also continuously eluted with the carrier and give rise to a constant detector signal when the capillary column is in an isothermal regime.

The bleeding rate depends on the oven temperature, carrier gas flow rate, stationary phase, quality of immobilization and crosslinking, amount and surface area of the stationary phase, and inertness of the capillary internal surface. Also, the rate of temperature programming can influence the bleed profile of a capillary column whenever higher rates increase column bleed.

After the 12-h conditioning periods with increasing final temperatures (250°C, 300°C, 325°C, 350°C, 370°C, and 390°C), bleeding observed for all capillary columns ranged from 12 pA for column 9 to 60 pA for columns 1 and 3. The higher bleeding observed in columns 1 and 3 was probably a result of the greater quantity of phenyl groups in their stationary phases.

Stability of the capillary external coating material

One important fact observed was the poor mechanical resistance of the aluminum-clad fused-silica capillary columns after the sequence of the Grob tests intercalated with 12-h conditioning periods with increasing final temperatures (250°C, 300°C, 325°C, 350°C, 370°C, and 390°C). After this set of experiments, columns 2 and 5 broke the extremities quite easily during capillary reinstallation in the chromatographic system. This behavior was consistent with that reported by Takayma et al. (9). It is important to realize that this does not hinder the use of this type of column, but it is necessary to be more careful and only remove the column when absolutely necessary.

A similar decrease of mechanical properties was also observed for column 3 (polyimide-coated fused-silica), but only after all experiments were realized. However, after the sequence of tests, column 1 was used for high-temperature mass spectrometry (MS) analysis, and after only 12 analyses (at up to 390°C and a final isotherm for 20 min), resolution problems were observed. When visually inspected, the column showed

degraded polyimide at several points of the capillary coil, exposing the fused-silica surface. The problems observed with column 1 could be attributed to faulty quality control of the polyimide material, because in our laboratory other columns from this manufacturer were previously used for high-temperature analyses and showed this behavior only after more than 100 runs. This was not observed with the other capillary columns evaluated. For instance, columns 7 and 9 were used for approximately 50 similar HT-HRGC–MS analyses each and showed no sign of polyimide-coating degradation.

The results showed that HT-HRGC commercial capillary columns could be used without serious problems at up to 420°C, but not with prolonged final isothermal at this temperature, because a temperature value of 420°C is still very limited. In fact, a neat distinction between HRGC and HT-HRGC is yet to be established, because in a few occasions, standard HRGC was used at up to 370°C (13) and 400°C (24). These problems with high-temperature capillary columns indicate that a renewed effort for the development of more stable and inert capillary columns would be necessary to push GC to its limit of 420°C.

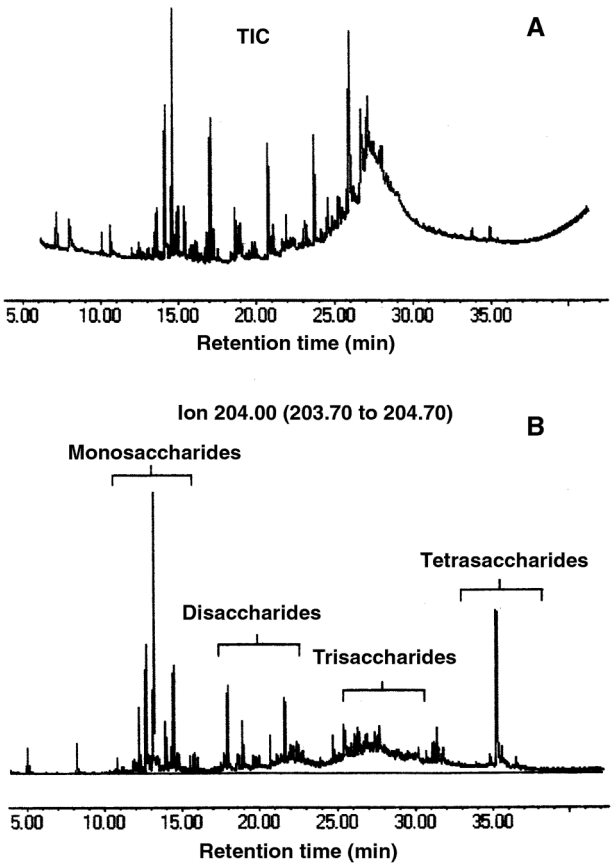

Figure 8. Total ion chromatogram of the crude methanolic extract of propolis sample A (found in Experimental section) (A) and a representative HT-HRGC mass fragmentogram (*m/z* 204) showing the distributions of saccharides (TMS derivatives) in the sample (taken from Sapucaia city, Rio de Janeiro, Brazil) and using column 1 programmed from 40°C at 8°C/min to 390°C and held for 20 min (B). FID and the on-column injector were operated at 400°C and room temperature, respectively. HT-HRGC coupled to MS analyses was performed on a HP 5972 MSD (Hewlett-Packard) under electron impact ionization (70 eV). The interface was at 320°C and the MS scan range was 40–700 daltons. (see Experimental section).

General considerations regarding high-temperature capillary columns

Grob in 1979 (25) discussed that one limitation of high-temperature capillary columns was the slow thermal degradation of the stationary phase under the catalytic influence of the support surface. It seemed that the problem can only be reliably overcome by using inert (i.e., leached and persilylated) surfaces (25). However, even though the activity of the columns was increased after sequences of conditioning at high temperatures, it was still possible to use these columns to obtain informative chromatograms (e.g., Figures 7 and 8). Adequate resolution was reached for high-molecular-weight compounds such as amyrin alkanoates (Figure 7) and complex mixtures of polar compounds (Figure 8).

Conclusion

The advent of HT-HRGC actually increased the upper temperature limit of HRGC by 40–60°C. This temperature difference at first sight seems minor, but in practice it is highly significant. Expressed in mass units of the compounds that can be analyzed, the working range can be extended at least by 400 daltons, and for certain structures, this represents analyses of up to 3000 daltons (5).

Commercial high-temperature capillaries can indeed be used at 390–420°C without major problems, except for a decrease in column lifetime.

This increase in the upper temperature limit that is now possible in routine analysis together with high inertness opens a new window for molecular mass that can be analyzed. This fact is very important for various fields of science (5), and the discovery of many new compounds can be envisaged (26–29).

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