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Triacylglycerol mixture for testing capillary columns for high-temperature gas chromatography

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Abstract

The quality of fused-silica capillary columns was investigated with a mixture of triacylglycerols at high temperatures. The molecular mass of the triacylglycerols ranges between 800 and 1140 and the elution temperatures are between 300 and 420°C. Essential properties of the capillary columns, such as separation power, selectivity, catalytic degradation, adsorption and bleeding were determined at high temperatures. Capillary columns coated with the five widely-used stationary phases OV-1, SE-52, OV-1701-OH, SOP-50 and OV-25 were checked. Elution patterns and information about separation power and degradation of the triacylglycerols are given. A simple method was elaborated to determine the column bleeding.

Keywords: Column bleeding; Column testing; Triacylglycerols; Lipids

1. Introduction

The quality of a gas chromatography separation system depends on various factors. The injector, the separation column and the detector are important for the separation and the quality of these parts has to be controlled. In particular, the separation columns have to be checked after preparation, intensive application and storage. The performance of the capillary column should not alter, however, if it does, changes should be seen with time. The reasons for deterioration in the quality of a capillary column are moisture and dirt introduced with the sample or with the carrier gas, high temperatures, oxygen and aging.

Some very helpful and generally used test mixtures for quality control are available. The most important and common one is the test mixture of Grob et al. [1,2]. This is a mixture of compounds with very different functional groups. The Grob test provides information about polarity, selectivity, separation efficiency and film thickness, for temperatures up to 150°C. Another important test is the Donike test [3], which consists of a mixture of alkanes and trimethylsilylesters of fatty acids with different chain lengths. The alkanes are thermally stable whereas the trimethylsilylesters of the fatty acids are very sensitive to catalytic degradation. The last trimethylsilyl (TMS) ester of the fatty acids elutes at around 300°C, therefore, the Donike test shows catalytic activities up to 300°C. The Donike test cannot be extended for use at higher temperatures, because fatty acids and alkanes with more than 36 carbon atoms are not widely available and solubility in organic solvents is very poor [4]. Both tests are very useful for moderate temperatures, but give no information about the quality of the capillary column at high temperatures.

Some attempts have been made to show the

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performance of capillary columns at higher temperatures: A mixture of an homologous series of polyethylene homopolymers (Polywax 500, 655 or 1000) is used to characterize columns [5–8]. The chromatograms give no information about the polarity of the stationary phase or about the degradation of the high boiling homologues at elevated temperatures, because the absolute concentrations of the individual compounds are not known.

The first reliable high-temperature test for capillary columns is the Triton test [4,9]. Triton X-100 [octylphenol–poly(ethylene glycol) ether] is partly methylated or ethylated and the other part is silylated with MSTFA. The peaks of the oligomers form a distribution curve and the heaviest oligomers elute at around 420°C. The main disadvantage of the Triton test is that it is not very sensitive to catalytic- and thermal degradation, because the silyl ethers are almost as stable as the alkyl ethers. Also, degradation is barely detectable because the content of each oligomer is unknown.

There are some reasons, why an alternative test for high-temperature capillary columns is required:

1. Separation efficiency may change with temperature. It is possible that capillaries do not work at higher temperatures because the separation power decreases with rising temperature. In contrast, the separation efficiency of capillary columns could also increase with rising temperature, i.e. when the minimum operating temperature is overstepped.
2. Selectivity changes with temperature: In particular, polarizable methylphenyl polysiloxanes have a changeable polarity [10,11]. The selectivity for double bonds and thus the polarity of the stationary phase increases with temperature.
3. Catalytic activities and adsorption effects of the column increase with temperature [3].
4. Thermally labile material decomposes at elevated temperature, even without catalytic effects.
5. Bleeding is an important property of high-temperature columns. It should be easy to measure the bleeding and the data obtained should be comparable to that obtained using other capillary columns. Bleeding rates are sometimes given in pico ampere (pA), but a more illustrative dimension would be useful, for example, the amount of bleeding products formed in 1 min and per 1 mg of stationary phase.

We developed, therefore, an alternative mixture for capillary columns for high-temperature GC (HTGC) using triacylglycerols with well known concentrations. Triacylglycerols are a perfect class of substances because they are components with high molecular masses and have widely varying compositions (an important field of research [10,12–17]); they are also sensitive to catalytic degradation and adsorption and are thermally labile [18]. Many triacylglycerols are commercially available, cheap and soluble in organic solvents.

The stationary phases tested consisted of common polysiloxanes, with the exception of SOP-50, which was specially synthesized for high temperatures [19]. Other commercially available capillary columns coated with polysiloxanes with phenyl or carborane groups in the siloxane backbone [7,20] were not investigated. These stationary phases would show excellent temperature stability (up to 480°C) but would have polarities as low as in the case of OV-1 or SE-52.

In this paper, we describe a method for checking the performance of capillary columns at high temperatures with a mixture of triacylglycerols. We present elution patterns for five widespread stationary phases and we have tabulated the relative responses of the triacylglycerols. Polarity, separation efficiency, catalytic activities and adsorption, thermal degradation and bleeding can be observed.

2. Experimental

2.1. Materials

All triacylglycerols were obtained from Sigma (St. Louis, MO, USA), except for trierucin (which was a gift from SHS, Heilbronn, Germany) and trilignocerin (which was obtained from Larodan, Malmö, Sweden). The purity of the compounds was at least 98%. The triacylglycerols were dissolved in chloroform in equivalent amounts per unit volume (=0.1 mg/ml). The abbreviations used for the triacylglycerols are given in Table 1.

The stationary phases OV-1 (100% dimethyl poly-

Table 1
Nomenclature of the triacylglycerols [12]

Number	Triacylglycerol	Abbreviation	Fatty acid	Molecular mass
1	Tripalmitin	PPP	3×C16:0	807.3
2	Tripalmitolein	PoPoPo	3×C16:1	801.3
3	Tristearin	SSS	3×C18:0	891.5
4	Triolein	OOO	3×C18:1	885.4
5	Trilinolein	LLL	3×C18:2	879.4
6	Trilinolenin	LnLnLn	3×C18:3	873.4
7	Triarachidin	AAA	3×C20:0	975.7
8	Trigadolin	GaGaGa	3×C20:1	969.6
9	Tribehenin	BeBeBe	3×C22:0	1059.8
10	Trierucin	ErErEr	3×C22:1	1053.8
11	Trilignocerin	LgLgLg	3×C24:0	1144.0
12	Trinervonin	NNN	3×C24:1	1137.9

siloxane) and SE-52 (5% phenyl, 95% methyl polysiloxane) were purchased from Chrompack (Middelburg, Netherlands). OV-1701-OH (88% methyl, 7% cyanopropyl, 5% phenyl polysiloxane) was obtained from Fluka (Buchs, Switzerland). The stationary phase, SOP-50, was provided by R. Aichholz (Ciba-Geigy, Basel, Switzerland) [19]. SOP-50 is a 50% dimethyl, 50% diphenyl polysiloxane and is symmetrically substituted and CH_3O-terminated. OV-25 (75% phenyl, 25% methyl polysiloxane) was obtained from Supelco (Bellefonte, PA, USA).

Fused-silica capillaries (0.32 mm I.D.) with a high-temperature polyimide coating were purchased from Polymicro Technologies (Phoenix, AZ, USA). The capillary columns (15 m length) were prepared using a procedure that has been described recently [21,22]. The capillaries were base-leached, acid-leached, rinsed, dehydrated, silylated with 1,3-dimethyl-1,1,3,3-tetraphenyl-disilazane and then were statically coated with a solution of the stationary phase resulting in a film thickness of 0.17 μm. The columns were conditioned up to 410 or 420°C.

2.2. Gas chromatography

All separations were performed on a Carlo Erba SFE 3060 gas chromatograph (Fisons, Rodano, Italy) equipped with a cold on-column injector, a flame ionization detector with a high-temperature stable ceramic flame jet and a constant pressure–constant flow module CP-CF 516 (Carlo Erba, Fisons, Italy). Hydrogen (99.999% pure) was used as the carrier gas. Data were recorded with a PE Nelson 3000 Series Chromatography Data System (Perkin-Elmer, Cupertino, CA, USA).

Cold on-column injection has to be preferred to split/splitless injection, because it is known to give better results with high molecular mass samples [23]. Analysis of triacylglycerols using PTV injection would also be practicable [24,25].

Triacylglycerol analyses were performed under constant flow regulation with a hydrogen linear velocity of 0.3 m/s. After cold on-column injection of 1 μl (=0.1 μg of each triacylglycerol) at 60°C, the oven was programmed to 280°C at 20°C/min and then to 410 or 420°C at 4°C/min. The final temperature was held isothermally for 10 min.

3. Results and discussion

Analyses of the triacylglycerol mixture give information about the separation power, catalytic activity and bleeding of the capillary columns at temperatures between 300 and 420°C. The elution order of the saturated and unsaturated triacylglycerols gives information about the selectivity of the stationary phase. The five saturated and the five triple unsaturated triacylglycerols form an homologous series that facilitates peak identification. The twelve components of the mixture are present in equal amounts. Therefore, peak areas and peak heights of the components should be identical, provided that the capillary column is absolutely catalytically inert. The relative responses make statements about the catalytic degradation of the different

components. The bleeding can be determined from the same measurement by a simple calculation, with no need for a derivatization step. The solution of triacylglycerols in chloroform is stable for several days if stored at ca. 4°C.

3.1. Selectivity

The elution order of saturated and unsaturated triacylglycerols and of the polyunsaturated triacylglycerols, trilinolein (LLL) and trilinolenin (LnLnLn), depends on the selectivity of the stationary phase.

The saturated triacylglycerols elute after the unsaturated triacylglycerols on apolar stationary phases (OV-1, SE-52). Fig. 1 shows an HTGC–flame ionization detection (FID) chromatogram of the triacylglycerol mixture separated on OV-1. The saturated and unsaturated components coelute on OV-1701-OH. The saturated triacylglycerols elute before the unsaturated triacylglycerols on the medium polar phases, SOP-50 and OV-25. An HTGC–FID chromatogram of the triacylglycerol mixture analyzed on OV-25 is given in Fig. 2.

The chromatograms of the triacylglycerol mixture separated on five different stationary phases are summarized as an elution pattern in Fig. 3. Some conclusions can be drawn from the elution patterns:

1. Elution temperatures of the triacylglycerols at standardized conditions decrease with increasing polarity of the stationary phase (for elution temperature of tripalmitin see Table 3). This observation agrees with the studies of Geeraert and Sandra [10].
2. Phenylmethyl silicones are known to be polarizable stationary phases. The selectivity for double bonds and, thus, the polarity of the phase increases with temperature [10,11]. Increases in the polarity of the stationary phases SOP-50 and OV-

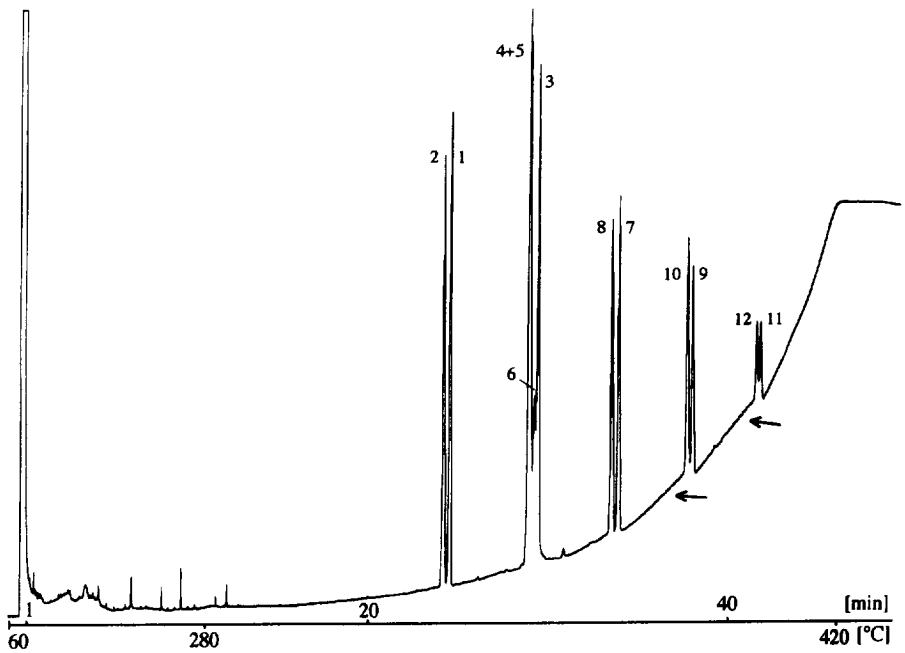

Fig. 1. HTGC–FID chromatogram of the triacylglycerol mixture separated on OV-1. For chromatographic conditions see Section 2. Peak identification: 1=PPP; 2=PoPoPo; 3=SSS; 4=OOO; 5=LLL; 6=LnLnLn; 7=AAA; 8=GaGaGa; 9=BeBeBe; 10=ErErEr; 11=LgLgLg and 12=NNN. For abbreviations, see Table 1.

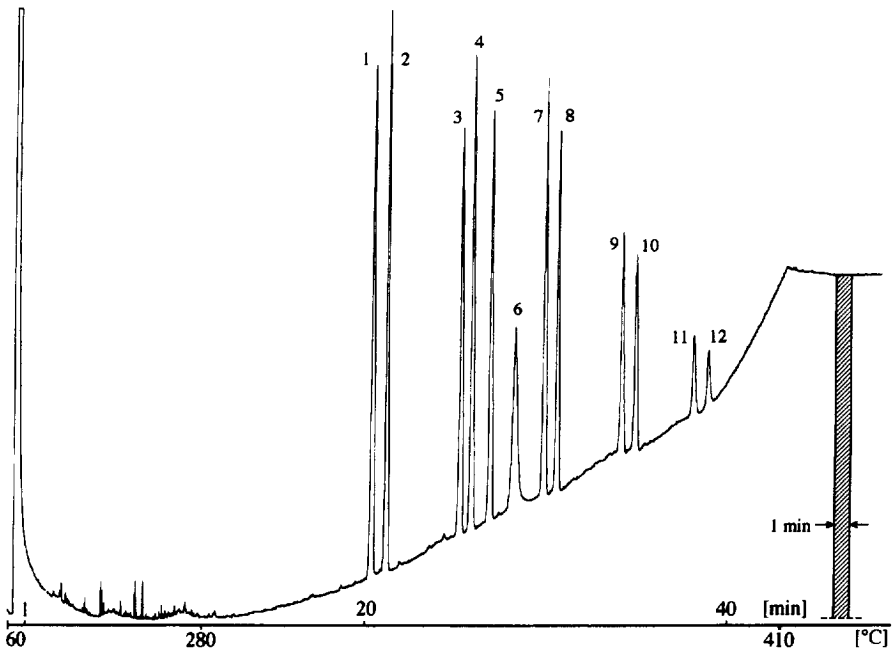

Fig. 2. HTGC–FID chromatogram of the triacylglycerol mixture analyzed on OV-25. For peak identification and chromatographic conditions see Fig. 1.

25 could be observed when the triacylglycerol analyses were performed using variable chromatographic conditions.

3.2. Resolution

The saturated and unsaturated pair, tripalmitin (PPP) and tripalmitolein (PoPoPo), (each with a carbon number of 48) was used to calculate the resolution [26].

The minus sign (in Table 2) indicates that PoPoPo elutes before PPP on apolar phases. The resolution is zero on OV-1701-OH, caused by coelution of PPP and PoPoPo.

3.3. Catalytic activity

The twelve triacylglycerols were used in equal amounts, therefore, all peaks should have the same areas if the capillary columns are catalytically inert. All of the capillary columns tested showed catalytic activities that increased with temperature.

The relative responses of the triacylglycerols separated on the five different stationary phases are given in Table 3. The peak area of PPP was set at 1.000. The peak areas of all other components are given relative to the value of PPP.

The chromatographic conditions, i.e., length and diameter of the capillary column, film thickness and carrier gas flow influence the elution temperature of the components and, therefore, highly influence the degree of degradation. The recovery of the components is improved with lower elution temperature (i.e., higher gas flow, shorter columns, slower temperature rate, thinner film thickness), but separation efficiency also decreases, as described in Ref. [17].

The relative response to the saturated and unsaturated triacylglycerols with the same carbon number is similar, however, the unsaturated triacylglycerols are usually more degraded, regardless of whether the

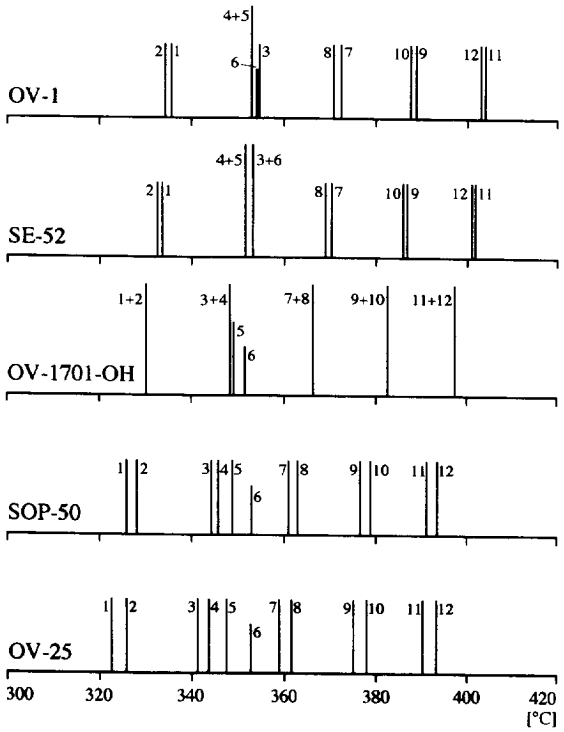

Fig. 3. Elution pattern of the triacylglycerol mixture of the five stationary phases OV-1, SE-52, OV-1701-OH, SOP-50 and OV-25. For peak identification and chromatographic conditions see Fig. 1.

unsaturated triacylglycerols eluted before or after the saturated triacylglycerols.

The polyunsaturated components with more than three double bonds, LLL and LnLnLn, give a measurement of the catalytic properties of the column. LnLnLn, in particular, does not show a good peak form and is degraded to a high degree.

In certain cases, the decomposition products of the triacylglycerols can be seen in the chromatogram (see arrows in Fig. 1). In particular, when the column is overloaded and at higher temperatures, the degradation products can be seen clearly. The base line increases shortly before the triacylglycerol elutes and decreases to a normal level after elution.

Table 2
Resolution of PPP and PoPoPo separated on five different stationary phases

Stationary phase	Resolution of PPP/PoPoPo
OV-1	−2.0
SE-52	−1.1
OV-1701-OH	0
SOP-50	3.3
OV-25	4.1

Calculation with coelution: if tristearin (SSS), triolein (OOO), LLL or LnLnLn coelute, a more convenient mixture is produced by using only two or three of these components. LLL and OOO, and, SSS and LnLnLn, coelute on SE-52. With only two components in the mixture, the single values of the relative response of each triacylglycerol can be determined. On OV-1701-OH, the saturated and unsaturated triacylglycerols coelute. For the purposes of calculation, the relative response value for PPP and PoPoPo is set at 200%.

Losses of material are not only caused by the separation column but also by the system used for sample introduction. The use of a cold on-column injector mainly prevents mass discrimination [23]. Nevertheless, a limit of the test mixture is that it is capable of distinguishing between losses produced by the injector or by the capillary column. Therefore, the whole chromatographic system is checked; not just the separation column.

3.4. Bleeding

The bleeding rate depends on the oven temperature, the carrier gas flow, the stationary phase, the quality of immobilization and crosslinking, and the amount and the surface area of the stationary phase.

The bleeding rate was determined using an idea by Schomburg et al. [27]. They used the *n*-alkane C22 to quantify the bleeding rate. Instead of the alkane, we used the triacylglycerol, PPP. The area and the known amount of PPP were compared to the area of the bleeding at the maximum allowable operating temperature (MAOT) formed in 1 min; $\text{mass}_{\text{bleeding}} = \text{area}_{\text{bleeding}} \times \text{mass}_{\text{PPP}} / \text{area}_{\text{PPP}}$ (see Fig. 2). The values of the capillary columns at MAOT are given in Table 4. The dimension of bleeding is μg of bleeding per time and amount of stationary phase.

These values are easy to determine, but do contain some systematic errors. Firstly, the response of the FID is not the same for the triacylglycerol and for the bleeding products. The response is 2.5 times higher for CH- than for Si compounds. Secondly, PPP is partly degraded, like all other triacylglyerols. Finally, the bleeding rate is proportional to the

Table 3
Recovery of the components of triacylglycerols separated on five different stationary phases and the elution temperature of PPP

Number	Triacylglycerol	OV-1	SE-52	OV-1701-OH	SOP-50	OV-25
1	PPP	1.000	1.000	2.000	1.000	1.000
2	PoPoPo	0.980	0.977		0.975	0.971
3	SSS	0.955	0.969	1.557	0.968	0.889
4	OOO	0.909	0.906		0.912	0.905
5	LLL	0.909	0.883	0.715	0.809	0.769
6	LnLnLn	0.712	0.765	0.517	0.753	0.673
7	AAA	0.781	0.839	1.253	0.874	0.773
8	GaGaGa	0.748	0.743		0.764	0.666
9	BeBeBe	0.484	0.551	0.644	0.639	0.466
10	ErErEr	0.550	0.565		0.594	0.430
11	LgLgLg	0.150	0.319	0.175	0.331	0.186
12	NNN	0.189	0.221		0.287	0.124
Elution temperature of PPP	336.0°C	333.2°C	330.4°C	325.6°C	322.2°C	

Table 4
Bleeding of different capillary columns at MAOT

Stationary phase	MAOT (°C)	Bleeding rate (μg/min mg)
OV-1	420	0.25
SE-52	420	0.11
OV-1701-OH	410	0.26
SOP-50	420	0.25
OV-25	410	0.31

surface area rather than to the amount of the stationary phase in the capillary column [27]. Nevertheless, the latter parameter was used because it is easily accessible. In spite of these limitations, the calculated values provide useful information about high-temperature capillary columns.

The data on the bleeding of the capillary columns coated with different stationary phases are quite similar, probably because the same column preparation procedure was used.

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