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STABLE AND REPRODUCIBLE SELECTIVE GLASS CAPILLARY COLUMNS WITH POLYSILOXANE STATIONARY PHASES FOR THE ANALYSIS OF FATTY ACID METHYL ESTERS

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SUMMARY

Efficient capillary columns containing OV-25, OV-225 and SP-2300 polysiloxane phases and minor additions of PEG 40M have been developed. The stationary phases were applied on the smooth dehydrated borosilicate glass walls of the capillaries by a high-pressure coating procedure. The reproducibility of column preparation has been determined. It was found that the selectivity of the columns remains the same during at least 1 year of use. The proposed columns are suitable for the identification of the individual components of complex mixtures of natural fatty acids.

INTRODUCTION

Earlier we proposed a method for the identification of long-chain fatty acid methyl esters (FAMEs) according to the equivalent chain length (ECL) data on four packed columns with polysiloxane phases of different polarity[1,2], which was used for the analysis of complex FAME mixtures of biological origin[3–5]. The use of open-tubular columns for the same purpose would have permitted an increase in the number of identifiable compounds owing to the higher separation efficiency.

In this work, the stability of the efficiency and selectivity of glass capillary columns with the polysiloxane phases OV-25, OV-225, SP-2300 containing minor additions of poly(ethylene glycol) (PEG) 40M during the long-term analysis of unsaturated FAMEs has been studied.

EXPERIMENTAL

Smooth dehydrated capillary walls of borosilicate glass (40 m × 0.25 mm I.D.) were coated with the stationary phases OV-25, OV-225 and SP-2300 with minor additions of PEG 40M by the high-pressure static procedure[6] under conditions described elsewhere[7]. The same method was used to prepare columns with OV-25, OV-225, SP-2300 and PEG 40M as individual stationary phases.

The columns were tested using a Pye Unicam 105 chromatograph equipped

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with a glass injector-splitter (1: 100). The column temperature was 200°C (OV-25, SP-2300, PEG 40M) and 210°C (OV-225) and the injector temperature was 250°C. The carrier gas was high-purity helium; the pressure drop was 1.50 atm.

Column selectivity was characterized by the ECL values[8] of natural unsaturated FAMEs with 1–6-methylene-interrupted double bonds in different positions. The standard mixture was a Baltic salmon FAME sample, which had been thoroughly studied in previous work[3]. To determine the ECL values the salmon FAME sample was injected together with methyl esters of normal saturated acids (14:0–24:0) in amounts that did not cause column overloading. The standard deviation of the ECL values over a total of 5-8 measurements did not exceed 0.03 ECL unit. The column efficiency at the carrier gas flow-rates close to the optimal values was determined by the theoretical plate numbers according to the broadening of the heneicosanoic acid methyl ester (21:0) zone. The standard deviation of the theoretical plate number of five measurements was within 200–300 theoretical plates per metre.

RESULTS AND DISCUSSION

Owing to their high selectivity with respect to methyl esters of natural long-chain fatty acids and their good thermal stability[9,10], polar polysiloxanes are the stationary phases most commonly used for the determination of the fatty acid content of lipids. However, the poor wettability of the smooth untreated glass surface by phenyl- and cyanopropylsiloxanes does not permit efficient capillary columns to be obtained by simple application of these stationary phases on the internal capillary walls. All the existing methods of preparing of such columns, involving a surface roughening stage (e.g., refs. 1 1–14) are multi-step procedures, which renders the preparation of columns with reproducible selectivity parameters very difficult. The peroxide-initited vulcanization of phenyl- and cyanopropylsiloxanes, which does permit highly efficient and stable capillary columns to be obtained, is suitable only for phases containing vinyl and tolyl substituents in the siloxane chain[15]. The reproducibility of selectivity parameters for columns with polar polysiloxane phases has generally not been reported.

In developing a procedure for the preparation of columns with reproducible selectivity we set ourselves the task of making it as simple as possible by reducing the number of steps involved. The following factors were taken into account: (1) FAMEs of biological origin except hydroxy acid esters do not show adsorption activity, and therefore no special deactivation of the glass capillary surfaces would be required; (2) high-temperature dehydration of the smooth glass surface improves its wettability by certain stationary phases' [6]; (3) application of a mixture of polyethylene glycol (Superox 20M) with a methylsiloxane elastomer (OV-1) yields highly efficient glass and fused silica capillary columns with smooth walls[17]; (4) addition of Superox4 to the polyether stationary phase SP-1000 increases the coating efficiency of fused-silica capillary columns with smooth internal surfaces preliminarily treated with Superox-4[18].

In this work, polysiloxane stationary phases with phenyl and cyanopropyl substituents of different composition were used: OV-25 (75% Phe), OV-225 (25% Phe, 25% CN) and SP-2300 (36% CN). These phases were applied together with small amounts of PEG 40M on to the smooth dehydrated internal surfaces of glass cap-

TABLE I

EFFICIENCY OF FRESHLY PREPARED GLASS CAPILLARY COLUMNS (THEORETICAL PLATES PER METRE) WITH OV-25, OV-225, SP-2300 CONTAINING AND NOT CONTAINING PEG 40M

The efficiency of the columns with OV-25 and SP-2300 was determined according to the broadening of the FAME 21:0 peak (200°C, capacity factor, $k = 3.0$) and of those with OV-225 according to the broadening of the FAME 20:0 peak (210°C, $k = 3.1$). Carrier gas, helium; pressure drop, 1.50 atm.

0 v-25		0 v-225		SP-2300	
Individual	*With PEG 40M*	*Individual*	*With PEG 40M*	*Individual*	*With PEG 40M*
2000	*3300*	*900*	*3200*	1700	3000

illaries. The resultant columns had an efficiency of 3000–3300 theoretical plates per metre and did not require any special conditioning prior to use.

The data in Table I permit a comparison of the initial efficiency of freshly prepared columns with *OV-25, OV-225* and SP-2300 containing and not containing additions of polyethylene glycol. It can be seen that the incorporation of a small amount of PEG 40M considerably improves the efficiency of smooth-walled capillary columns. The quality of columns that did not contain polyethylene glycol deterio-

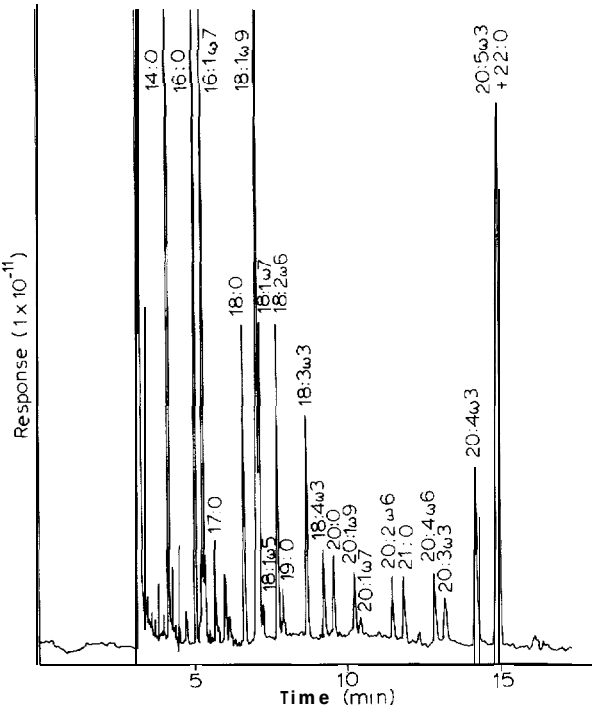

Fig. 1. Part of the chromatogram of the separation of the mixture of Baltic salmon FAMEs and saturated 14:0–24:0 FAMEs on a 40 m × 0.25 mm I.D. glass capillary column with SP-2300 containing a small amount of PEG 40M. Temperature of analysis, 200°C; carrier gas, helium; pressure drop, 1.50 atm.

TABLE II

REPRODUCIBILITY OF THE ECL VALUES OF UNSATURATED FAME ON FRESHLY PREPARED CAPILLARY COLUMNS WITH SP-2300 AND A SMALL AMOUNT OF PEG 40M

Temperature of analysis, 200°C; carrier gas, helium; pressure drop, I .50 atm. The standard deviation of ECL values over a total of 5–8 measurements was found to be within 0.03 unit.

*FAME**	Equivalent *chain length* (ECL)			
	Column I	*Column* 2	Column 3	Column 4
18:2ω6	18.84	18.85	18.86	18.86
18:3ω3	19.45	19.47	19.47	19.48
18:4ω3	19.76	19.79	19.78	19.79
20: 109	20.31	20.32	20.32	20.32
20:2ω6	20.83	20.84	20.82	20.85
20:3ω3	21.44	21.47	21.45	21.47
20:4ω3	21.76	21.78	21.77	21.77
20:4ω6	21.33	21.36	21.33	21.35
20:5ω3	21.97	21.99	21.97	21.96
22:6ω3	24.17	24.18	24.16	24.17

* In the FAME notation, the first figure indicates the number of carbon atoms in the acid chain, the second the number of methylene-interrupted double bonds and the ω value shows the position of the last double bond relative to the terminal methyl group.

rated rapidly during the first few days in use, e.g., the efficiency of columns without PEG 40M dropped to 900–600 theoretical plates per metre in 3 days. After that period, with SP-2300 one could observe rare but large accumulations which were, apparently, coalescent drops of the stationary phase. In contrast, the columns with PEG 40M added to the stationary phase retained an efficiency of 3000 theoretical plates per metre for over 1 year. It can be assumed that even in small amounts PEG

TABLE III

ECL VALUES OF UNSATURATED FAME DURING EXTENDED USE OF A CAPILLARY COLUMN WITH SP-2300 AND A SMALL AMOUNT OF PEG 40M

Temperature of analysis, 200°C; carrier gas, helium; pressure drop, 1.50 atm. The standard deviation of ECL values over a total of 558 measurements did not exceed 0.03 unit.

FAME	Period of use of column (months)				
	0	3	6	9	12
20: 1ω9	20.31	20.31	20.31	20.32	20.33
18:2ω6	18.84	18.86	18.84	18.85	18.87
20:2ω6	20.83	20.83	20.83	20.85	20.84
18:3ω3	19.45	19.45	19.46	19.46	19.48
20:3ω3	21.44	21.45	21.44	21.46	21.46
18:4ω3	19.76	19.80	19.77	19.78	19.80
20:4ω3	21.76	21.76	21.75	21.78	21.76
20:4ω6	21.33	21.32	21.31	21.33	21.34
20:5ω3	21.97	21.94	21.94	21.95	21.95
22:6ω3	24.17	24.15	24.16	24.17	24.15

TABLE IV

ECL VALUES OF UNSATURATED FAME ON GLASS CAPILLARY COLUMNS WITH OV-25, OV-225 AND SP-2300 CONTAINING AND NOT CONTAINING PEG 40M AND ON A COLUMN WITH INDIVIDUAL PEG 40M

Temperature of analysis, 200°C (OV-25, SP-2300, PEG 40M) and 21O'C (OV-225); carrier gas, helium; pressure drop, 1.50 atm. The standard deviation of ECL values was within 0.03 unit.

FAME	0 v-25			0 v-225			*SP-2300*			*PEG 40M (pure)*
	With PEG 40M	*Without PEG 40M*	*AECL*	*With PEG 40M*	*Without* PEG 40M	*AECL*	*With PEG 40M*	*Without* PEG 40M	*AECL*	
18:2ω6	18.31	18.32	−0.01	18.45	18.47	-0.02	18.84	18.82	+ 0.02	18.47
18:3ω3	18.65	18.65	0.00	18.86	18.87	-0.01	19.46	19.43	+ 0.03	19.00
18:4ω3	18.75	18.75	0.00	–	–	–	19.77	19.71	+ 0.06	19.24
20:1ω9	20.07	20.08	-0.01	20.11	20.13	*-0.02*	20.31	20.30	+ 0.01	20.08
20:2ω6	20.30	20.30	0.00	20.43	20.43	*0.00*	20.83	20.81	+ 0.02	20.36
20:3ω3	20.64	20.62	+ 0.02	20.87	20.87	*0.00*	21.44	21.41	+ 0.03	20.81
20:4ω3	20.74	20.71	+ 0.03	–	–	–	21.75	21.71	+ 0.04	21.00
20:4ω6	20.39	20.36	+ 0.03	20.62	20.63	-0.01	21.31	21.27	+ 0.04	20.69
20:5ω3	20.74	20.71	+ 0.03	21.04	21.01	*+ 0.03*	21.94	21.88	+ 0.06	21.20
22:5ω3	22.72	22.68	+ 0.04	–	–	–	23.97	23.91	+ 0.06	23.00
22:6ω3	22.79	22.75	+ 0.04	23.10	23.14	*-0.04*	24.16	24.08	+ 0.08	23.23

40M, which exhibits the properties of a high-molecular-weight surfactant, improves the wettability of glass and promotes a uniform distribution of the liquid phase over the wall surface, thereby ensuring a high efficiency of the columns. The mechanism is probably more complex and includes partial adsorption of polyethylene glycol molecular segments by the capillary walls and stabilization of the liquid phase layer due to the intermolecular interactions.

It is known that the more polar the stationary phase is, the harder it is to obtain capillary columns that are reproducible with respect to the selectivity parameters and stable in use. Therefore, we used the most polar of the selected phases, SP-2300, as a model for the investigation of the reproducibility and stability of columns. The reproducibility of columns was determined by the ECL values of natural FAMEs with different numbers of double bonds. A fragment of the chromatogram of the FAME mixture used for column testing is shown in Fig. 1. Table II gives the ECL values of natural FAMEs containing 1-6 double bonds measured on columns with SP-2300 prepared at different times from different batches of borosilicate glass. From Table II, it follows that even for polyunsaturated FAMEs, which are most sensitive to selectivity changes, the variation of ECL values for different columns was within 0.01–0.03 unit, which is less than the experimental error.

The stability of columns with SP-2300 and the addition of PEG 40M was monitored over a period of 1 year by periodic testing. It can be seen from Table III that the variation of the ECL values with time is definitely stochastic, independent of the degree of FAME unsaturation, and is within the error of the ECL determination. This means that columns retain an unchanged selectivity over a long period of use. The high efficiency and the stable and reproducible selectivity of the resultant columns allows them to be used for identification of the FAMEs in complex mixtures of natural origin.

Testing of the column with pure PEG 40M as an individual stationary phase showed that as regards selectivity with respect to unsaturated FAMEs PEG 40M is closest to OV-225, and in this respect is less polar than SP-2300 but more polar than OV-25. One could expect, therefore, that the addition of minor amount of PEG 40M would either leave the ECL values typical for individual stationary phases almost unchanged, or would increase them with OV-25 and decrease them with SP-2300. As can be seen from Table IV, addition of polyethylene glycol does indeed slightly increase the polarity of OV-25 and has almost no effect on the polarity of OV-225. However, the addition of PEG 40M to SP-2300 does not decrease but unexpectedly increases the polarity of the latter. For example, the increase in ECL values (ΔECL) for FAMEs containing five and six double bonds due to addition of polyethylene glycol to SP-2300 is 0.06–0.08 unit. When the amount of PEG 40M added is doubled, the increase in ECL values observed is 0.15–0.18 unit. Hence, the effect of addition of PEG 40M on the selectivity parameters depends on the chemical nature of the basic stationary phase. Apparently, addition of polyethylene glycol to the cyanopropyl siloxane SP-2300 involves processes that alter its physico-chemical properties considerably.

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