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SHORT CAPILLARY COLUMNS IN THE ANALYSIS OF LIPIDS

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SUMMARY

Relatively short capillary columns have been employed for the gas–liquid chromatographic separation of lipid components for two reasons: to reduce the time of instrumental analysis while maintaining the degree of separation typical of packed columns, and to obtain better results more quickly as a result of faster elution. In this study, several elutions of naturally occurring normal and rare fatty acids, unsaponifiable components, mixtures of glycerides and related problems are discussed.

INTRODUCTION

Gas–liquid chromatography (GLC) with "short" capillary columns seems likely to find widespread application in the future. The advantage of capillary columns over the traditional packed columns is that they allow one to obtain comparable results within a shorter time, because the elution time is approximately proportional to the inverse of the column length.

In 1976, Prevot and Mordret[1] reported several uses for short capillary columns in the analysis of lipid components. A 15-m glass column coated with Carbowax 20M is long enough to allow the separation of stearic and oleic acids (as methyl esters) and to elute in a few minutes the methyl ester mixture, which shows the presence of behenic acid (n-$C_{22:0}$). The advantage of using this kind of GLC determination in routine analysis is evident. Moreover, the high sensitivity of the detectors suitable for capillary column GLC makes it possible to analyse very small samples.

EXPERIMENTAL AND RESULTS

A new application of short capillary columns that use the same experimental conditions (column, temperature programme, on-column injection) needed for glyceride elution[2] is total lipid analysis carried out on blood serum. Fig. 1 shows a characteristic GLC trace of this natural mixture obtained by the use of a 10-m glass column coated with SE-52. Recently, this rapid method, which allowed the simultaneous evaluation of serum lipid parameters (triglycerides, TG; cholesteryl esters, CE; free cholesterol, C; free fatty acids, FFA; etc.) allowed a nutritional study to be carried out on rats[3].

Fig. 2 shows the chromatogram of the separation of fatty acids (as methyl

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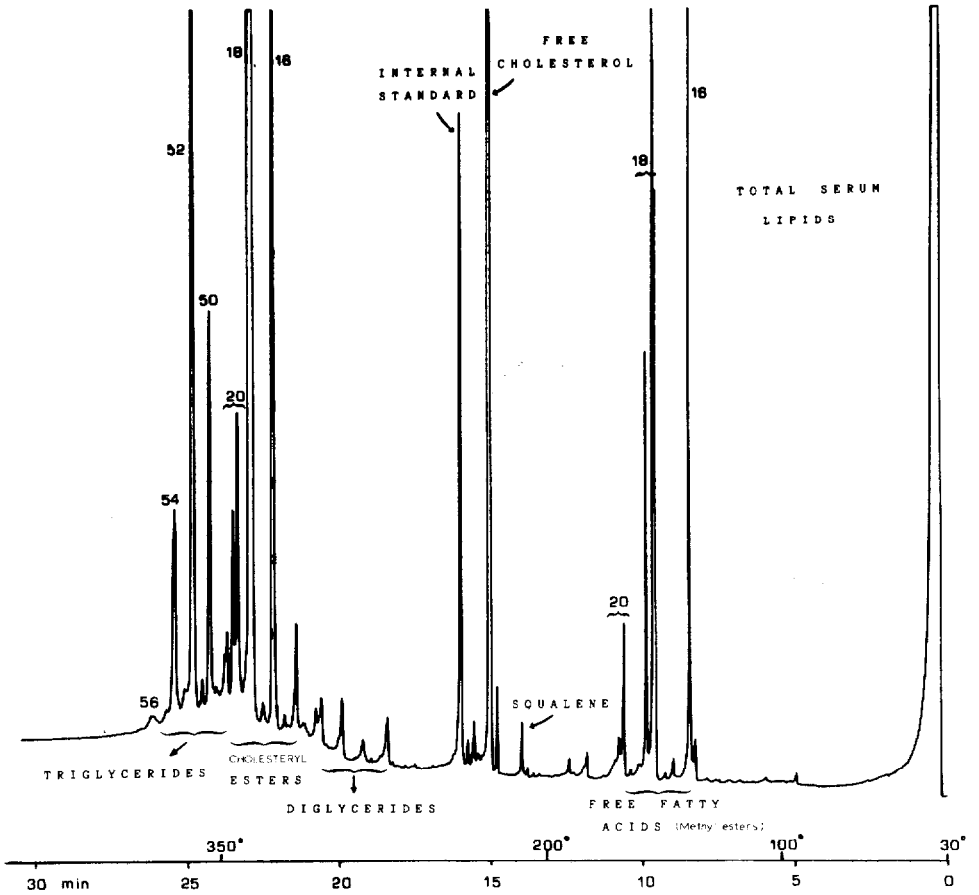

Fig. 1. Gas chromatogram of serum lipids after treatment with diazomethane, obtained using a 10-m glass column coated with SE-52. Column temperature: programmed from 30 to 350°C, at 13°C/min. On-column injection system (Grob). Carrier gas (helium) flow-rate: 2.5 ml/min.

esters) in rapeseed oil with a high erucic acid content on a 15-m fused-silica column coated with Carbowax 20M. The few minutes required for the separation contrast with the elution times characteristic of DEGS or similar packed columns (about 20–30 min). Under similar conditions, it is also possible to elute the fatty acids in castor oil (as methyl esters) in a relatively short time. The result, shown in Fig. 3, again cannot be obtained as quickly with packed columns. With castor oil, however, as it is necessary to show hydroxymethyl esters (ricinoleate and dihydroxystearate), the column temperature must be increased.

The separation of the organic acids in royal jelly (as methyl esters) is shown in Fig. 4. A 20-m glass column coated with OV-17 gave within a few minutes the best separation compared with other stationary phases or packed columns[4]. Royal jelly contains in its lipid fraction (which is 4–12% of the dry matter) almost 90% of free organic acids (linear and branched-chain hydroxy fatty acids, dicarboxylic acids, fatty acids and dihydroxy fatty acids).

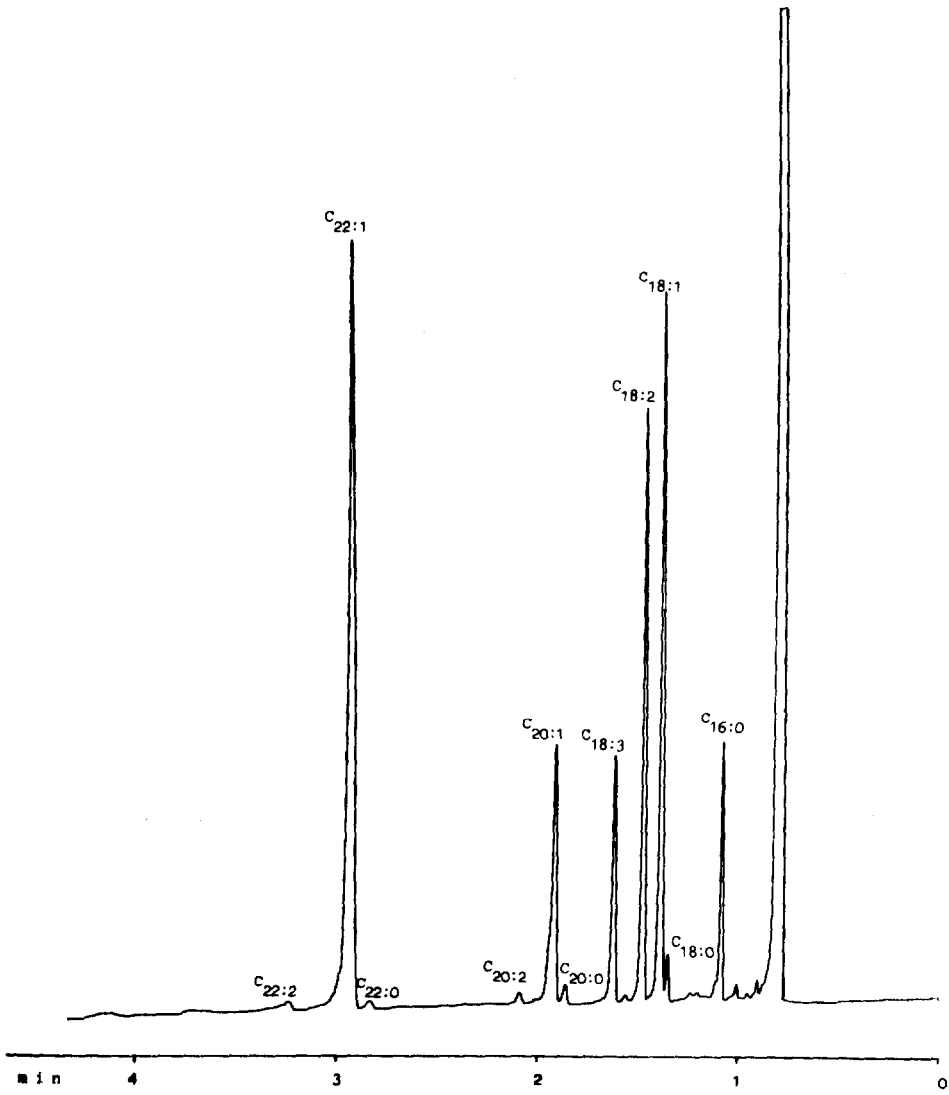

Fig. 2. Gas chromatogram of methyl esters of fatty acids of repeseed oil with a high erucic acid content, obtained using a 15-m fused-silica column coated with Carbowax 20M. Column temperature: 200°C. Split injection (splitting ratio 1:80). Carrier gas (helium) flow-rate: 1.5 ml/min.

The presence of fatty acids with a cyclopropane ring (malvalic acid, dihydrosterculic acid, etc.) in malva seed oil (*Abutilon avicennae*, Gaertn.) was shown by use of a 25-m glass column coated with OV-1 (Fig. 5). Under these conditions, it appears that very small amounts of this oil can be detected.

The GLC of the unsaponifiable fractions of lipid substrates (obtained by preparative thin-layer chromatography) also has diagnostic uses[5]. That columns of different polarity can be used for sterol determination, *e.g.*, short capillary columns coated with OV-17 (or free fatty acid phase, FFAP) and silicones (SE-30, SE-52,

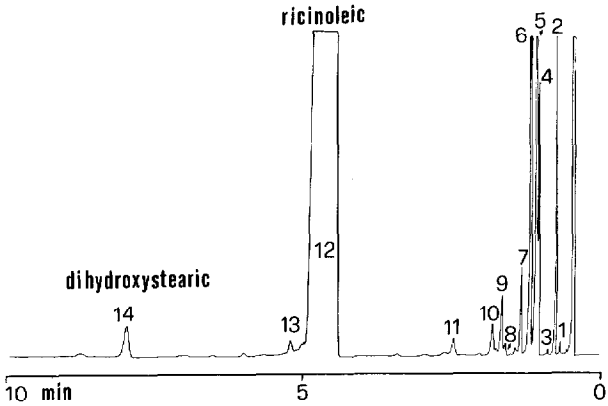

Fig. 3. Gas chromatogram of fatty acids of castor oil (as methyl esters). Conditions as in Fig. 2. Peaks: 1 = pentadecanoic acid; 2 = palmitic acid; 3 = margaric acid; 4 = stearic acid; 5 = oleic acid; 6 = linoleic acid; 7 = linolenic acid; 8 = arachidic acid; 9 = eicosenoic acid; 10 = eicosadienoic acid; 11 = behenic acid, 13 = unknown.

SE-54, SF-96, etc.), is due to the molecular characteristics of these cyclic alcohols.

Fig. 6 shows the GLC trace obtained for the sterols in tomato seed oil. In this instance, the greater polarity of the FFAP column gives a better separation of the peaks occurring after β-sitosterol (peak 10 in Fig. 6). The elution time (8 min) is very short in comparison with the time needed for packed column elution (about 60 min).

The use of high-boiling point stationary phases, such as SE-52, allows one to detect the "dimeric" components present in the peroxidation of methyl oleate (Fig. 7)[6]. In fact, the hydroperoxy and hydroxy radicals arising from methyl oleate per-

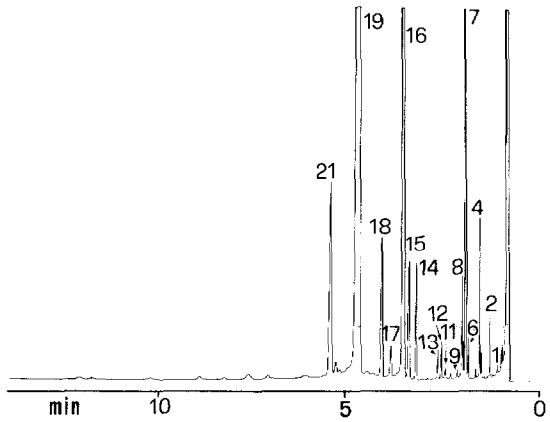

Fig. 4. Gas chromatogram of free organic acids of royal jelly (as methyl esters), obtained using a 20-m glass column coated with OV-17. Column temperature: 160°C. Splitter injection (splitting ratio 1:100). Carrier gas (helium) flow-rate: 2.5 ml/min. Peaks: 4 = 7-hydroxyheptanoic acid; 7 = 8-hydroxyoctanoic acid; 9 = octanedioic acid; 11 = 9-hydroxynonanoic acid; 12 = methyloctanedioic acid; 13 = 9-hydroxydecanoic acid; 14 = methyloctenedioic acid; 15 = 9-hydroxydecenoic acid; 16 = 10-hydroxydecanoic acid; 18 = decanedioic acid; 19 = 10-hydroxydecenoic acid; 21 = decenedioic acid.

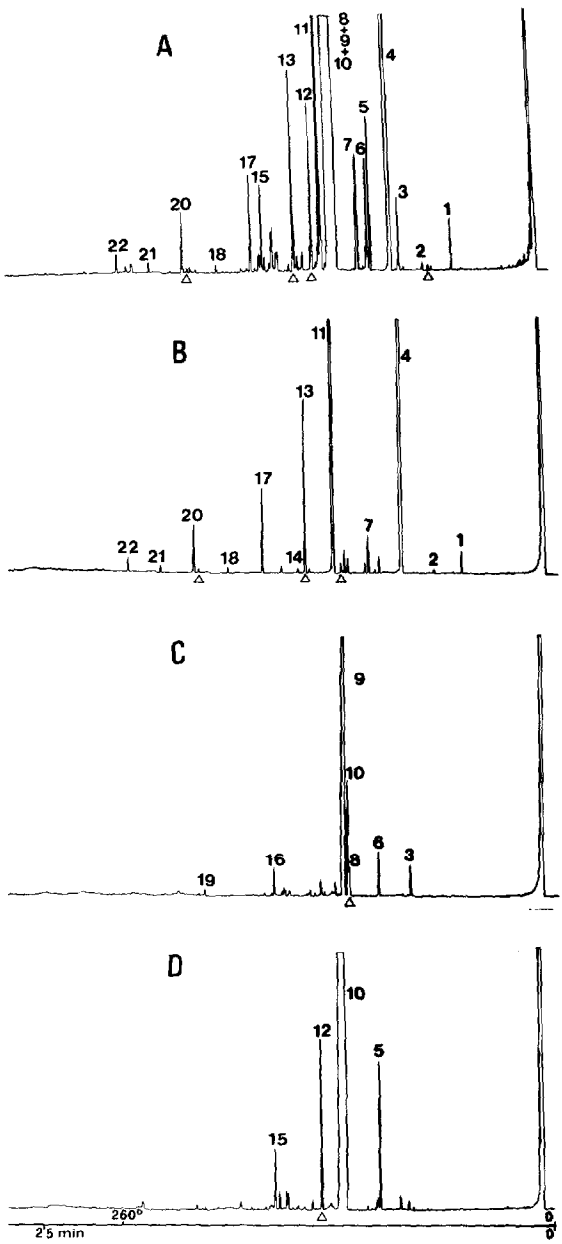

Fig. 5. Gas chromatogram of the preparative thin-layer chromatographic (silver nitrate) fractions of fatty acids (as methyl esters) of malva oil (*Abutilon avicennae*, Gaertn.), obtained using a 25-m glass column coated with OV-1. Column temperature: programmed from 150 to 260°C at 5°C/min. Split injection (splitting ratio 1:80). Carrier gas (helium) flow-rate: 2.2 ml/min. Traces: (A) total mixture; (B) saturated; (C) monounsaturated; (D) diunsaturated fractions. Peaks: 1 = $C_{14:0}$; 2 = $C_{15:0}$; 3 = $C_{16:1}$; 4 = $C_{16:0}$; 5 = $C_{17:2}$; 6 = $C_{17:1}$; 7 = $_{17:0}$; 8 = $C_{18:1}$ cyclo; 9 = $C_{18:1}$;10 = $C_{18:2}$; 11 = $C_{18:0}$; 12 = $C_{19:2}$ cyclo; 13 = $C_{19:0}$ cyclo; 14 = $C_{19:0}$; 15 = $C_{20:2}$; 16 = $C_{20:1}$; 17 = $C_{20:0}$; 18 = $C_{21:0}$; 19 = $C_{22:1}$ cyclo; 20 = $C_{22:0}$; 21 = $C_{23:0}$; 22 = $C_{24:0}$. Δ = cyclopropane ring in the molecule.

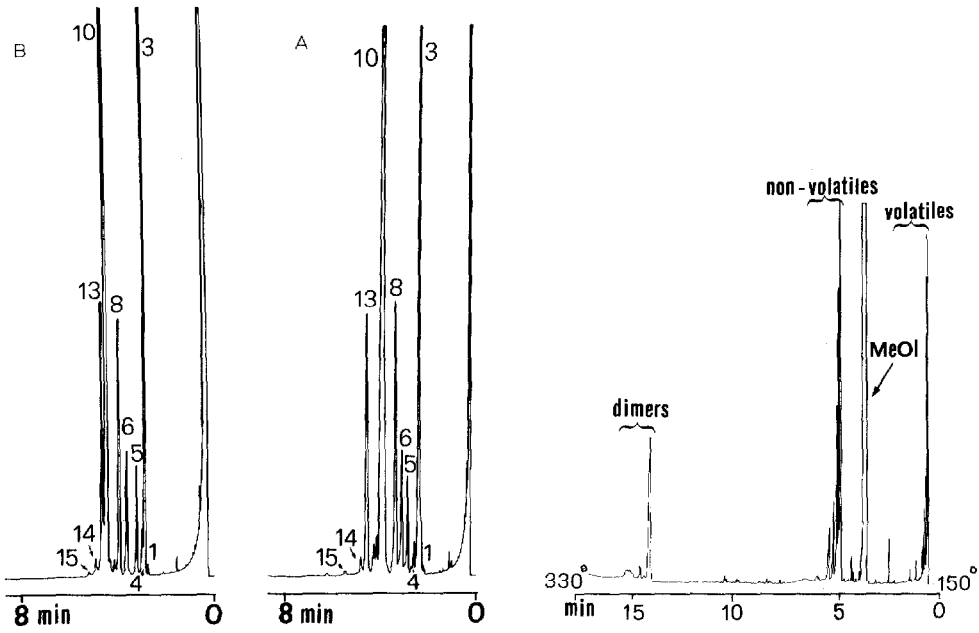

Fig. 6. Gas chromatogram of the sterols of tomato seed oil. (A) 9-m glass column coated with FFAP. Column temperature: 205°C. Split injection (splitting ratio 1:100). Carrier gas (helium) flow-rate: 2.5 ml/min. (B) 10-m glass column coated with SE-30. Column temperature: 250°C. Split injection (splitting ratio 1:100). Carrier gas (helium) flow-rate: 2.5 ml/min. Peaks: 1 = dehydrocholesterol (tentative); 3 = cholesterol; 4 = 24-methylenecholesterol; 5 = brassicasterol; 6 = campesterol; 8 = stigmasterol; 10 = β-sitosterol; 13 = Δ^5-avenasterol; 15 = Δ^7-stigmastenol.

Fig. 7. Gas chromatogram of the pyrolysis of peroxidized methyl oleate, obtained using a 10-m glass column coated with SE-52. Column temperature: programmed from 150 to 330°C at 8°C/min. Split injection (splitting ratio 1:60). Carrier gas (helium) flow-rate: 2.5 ml/min. MeOl = methyl oleate (substrate).

oxidation carried out at high temperatures produce small amounts of compounds with molecular weights approximately double that of the substrate.

CONCLUSIONS

The adoption of short capillary columns in the analysis of lipids, which at present is employed only for research purposes, should spread quickly, for two main reasons: the high quality of available instruments (gas chromatographs, columns, calculators, computers, etc.) and the shorter time of elution and increased resolution. Both of these factors contribute to excellent results in routine analysis.

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