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ON-COLUMN INJECTION IN THE CAPILLARY GAS CHROMATOGRAPHIC ANALYSIS OF FATS AND OILS

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SUMMARY

A custom-built, movable on-column injection device in which the injection point is cooled outside the oven during injection is described. In this way, injections can be performed at high oven temperatures, simplifying routine capillary gas chromatographic analysis of high-boiling compounds. The possibilities of this approach of on-column injection are illustrated with the analysis of fatty acid methyl esters, fats and oils and ozonized fats and oils.

INTRODUCTION

The progress made in high-temperature capillary gas chromatography (GC) is mainly due to the development of high-temperature silylated non-polar columns[1,2] and the introduction of cold on-column injection[3,4]. This opened the way for the routine analysis of complex high-molecular-weight samples, such as triglycerides. A serious drawback of commercially available injection devices for high-temperature work is the need for cooling of the oven below the boiling point of the solvent or, when secondary cooling is available, to 10–30°C above the boiling point of the solvent. To overcome this problem, a movable on-column injector was built, making the injection zone temperature independent of the column oven temperature. The idea of removing the injection zone of the capillary column oven, *i.e.*, of applying natural cooling, is not new and has been patented by Carlo Erba. Takayama[5] also described a natural cooling column system without stressing high-temperature work.

The movable on-column injector device described in this paper has been in use for more than 2 years for high-temperature work. The main advantages of the system are its simplicity, the precise and accurate results obtained, and the gain of time in routine capillary GC.

The possibilities of the injector are illustrated with several examples of fat and oil analyses.

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EXPERIMENTAL

On-column injection device

The injector is made from a 1/4–1/16-in. Parker reducing union, through the base of which a 1/16-in. stainless-steel tube is inserted and silver-soldered for supplying the carrier gas. The glass capillary is connected to the 1/16-in. end, and is guided via a 1/8-in. vespel ferrule placed in the 1/4.-in. part of the union. In the vespel ferrule a second bore is drilled to allow the carrier gas to enter the column. The 1/4-in. nut is equipped with a needle guide, and a duck-bill valve is inserted to isolate the chromatographic system. The injector has a total height of 4 cm and is connected to a vertical slider, allowing the injector to move 10 cm up and down with respect to the column oven wall. A drawing of the injection device and full details of its operation are described in ref. 18.

Capillary gas chromatography

The injection device was mounted in a Hewlett-Packard 5880 A gas chromatograph, equipped with a flame-ionization detector. The glass capillary columns (5 and 15 m long, 0.3 mm I.D.) were prepared from soda-lime glass. The columns were leached at 150°C successively with 18% hydrochloric acid and 0.1% hydrofluoric acid. Dehydration was performed at 300°C. High-temperature silylation was carried out at 400°C with pure hexamethyldisilazane (HMDS). The columns were statically coated with a 0.1% solution of OV-1 in *n*-pentane. The columns were further stabilized by introduction of gaseous HMDS during the conditioning period (72 h repeatedly from 200 to 370°C at 2°C/min). Peak integration and calculation were performed on a Hewlett-Packard 3353 E Lab Data System.

Nomenclature

The following abbreviations are used.

Co	caproic acid, hexanoic acid, C 6:0.
Cy	caprylic acid, octanoic acid, C 8:0.
C	capric acid, decanoic acid, C 10:0.
La	lauric acid, dodecanoic acid, C 12:0.
M	myristic acid, tetradecanoic acid, C 14:0.
P	palmitic acid, hexadecanoic acid, C 16:0.
S	stearic acid, octadecanoic acid, C 18:0.
A	arachidic acid, eicosanoic acid, C 20:0.
Be	behenic acid, docosanoic acid, C 22:0.
O	oleic acid, *cis*-9-octadecenoic acid, C 18:1.
L	linoleic acid, *cis,cis*-9,12-octadecadienoic acid, C 18:2.
Ln	linolenic acid, *cis,cis,cis*-9,12,15-octadecatrienoic acid, C 18:3.
G	gadoleic acid, *cis*-11-eicosenoic acid, C 20:1.
Er	erucic acid, *cis*-13-docosenoic acid, C 22:1.
U	unsaturated fatty acid with 18 carbon atoms.
$\Psi(x)$	fatty acid cleaved at position x.

RESULTS AND DISCUSSION

The characteristics of the injection device were evaluated with the analysis of fats and oils.

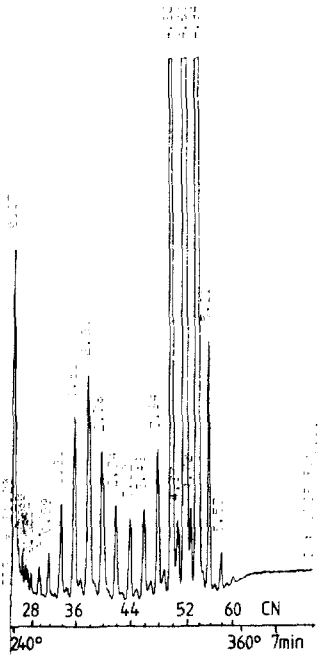


Fig. 1. Fast carbon number separation of milk chocolate fat triglycerides.

Rapid separations of triglycerides according to carbon number by capillary GC were described by Mordret *et al.*[6] and D'Alonzo and co-workers[7,8]. Fig. 1 shows the analysis of the triglycerides of a milk chocolate in about 6 min on a 5-m capillary column, when 0.04 μl of a 0.1 % milk chocolate fat solution in isooctane was introduced with a 0.5-μl syringe via the on-column device described above. The oven temperature during injection was 240°C. After the solvent had passed the detector, the injector zone was pushed into the GC oven and the temperature was programmed at 20°C/min to 360°C. Compounds with even and odd carbon numbers from C_{28} to

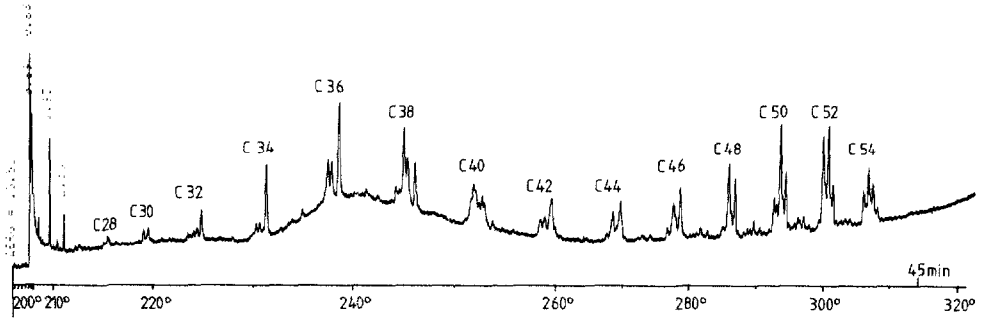


Fig. 2. Analysis of butterfat by capillary GC. Sample, 0.1 μl of 0.02 % butterfat in *n*-pentane; column, 15 m × 0.3 mm I.D. HTS-OV-1; oven temperature, 200°C, 1 min isothermal, 10°C/min to 210°C, 2°C/min to 260°C, 3°C/min to 320°C; carrier gas, hydrogen, 0.22 bar.

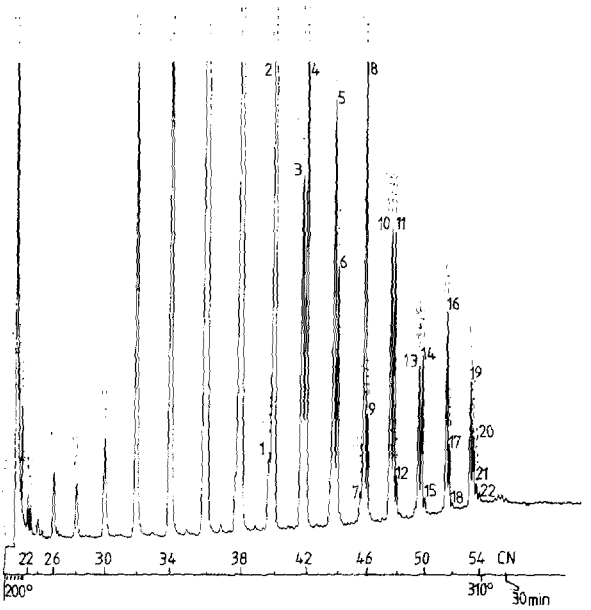
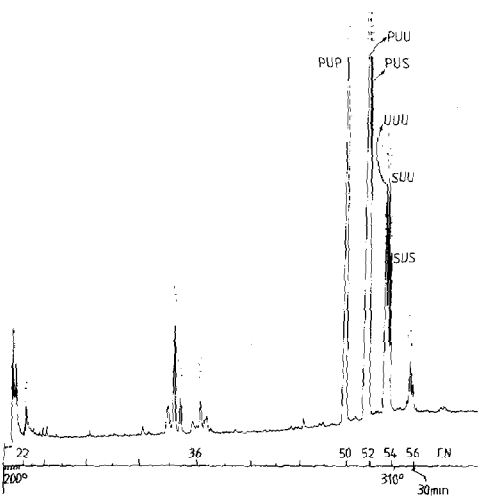


Fig. 3. Analysis of palm kernel oil by capillary GC. Sample, 0.04 μl of 0.3 % palm kernel oil in isooctane; column, as in Fig. 2; oven temperature, 200°C, 1 min isothermal, 4°C/min to 310°C, 2 min isothermal; carrier gas, hydrogen, 0.3 bar. Tentative identification of fine structure of C_{40}–C_{54} peaks: C_{40}, (1) CyMU + CoPU, (2) LaMM + LaLaP + CMP + CyMS + CoPS; C_{42}, (3) LaLaU + CMU + CyPU, (4) LaMP + CPP + LaLaS + CMS + CyPS; C_{44}, (5) LaMU + CPU + CySU, (6) MMP + LaPP + LaMS + CPS + CySS; C_{46}, (7) CUU, (8) LaPU + CSU, (9) MPP + LaPS + CSS; C_{48}, (10) LaUU, (11) MPU + LaSU, (12) PPP + MPS + LaSS; C_{50}, (13) MUU, (14) PPU + MSU, (15) PPS + MSS; C_{52}, (16) PUU, (17) PSU, (18) PSS; C_{54}, (19) UUU, (20) SUU, (21) SSU, (22) SSS.



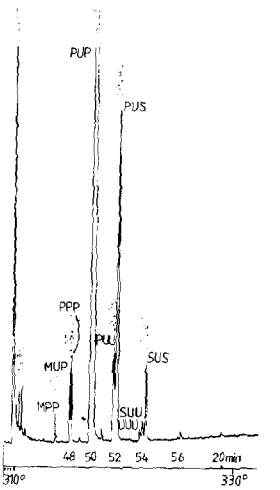
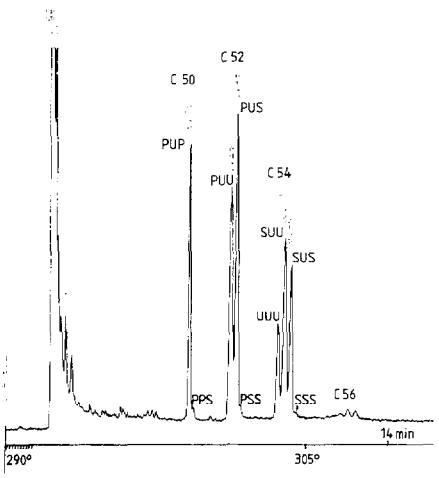


Fig. 4. Analysis of coffee oil by capillary GC. Conditions as in Fig. 3.

Fig. 5. Analysis of palm oil by capillary GC. Sample, 0.1 μl of palm oil midfraction in pentane; column, as in Fig. 2; oven temperature, 310°C, 1 min isothermal, 1°C/min to 330°C; carrier gas, hydrogen.



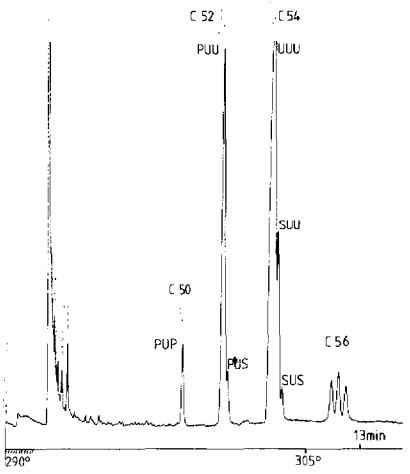


Fig. 6. Analysis of mowrah oil by capillary GC. Sample, 0.05 μl of 0.01 % mowrah oil in isooctane; column as in Fig. 2; oven temperature, 290°C, 1 min isothermal, 1.5°C/min to 305°C, 3 min isothermal.

Fig. 7. Analysis of peanut oil by capillary GC. Sample, 0.05 μl of 0.01 % peanut oil in in isooctane; conditions as in Fig. 6.

C_{58} were easily resolved. This analysis was used for the accurate quantitation of milk fat in cocoa butter[9] and for checking the purity of cocoa butter[10]. Rapid profiling was also used for the preliminary examination of unknown fat samples.

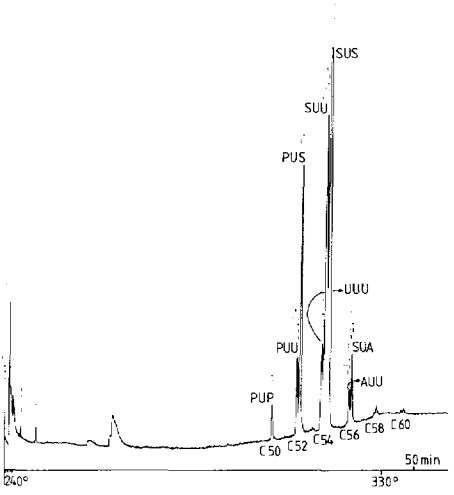
More refined separations, showing the fine structure of the carbon number peaks were obtained on the 15-m column. Separations of triglycerides, showing different degrees of fine structure, have been published by Monseigny *et al.*[11], Grob[12], Riva and Galli[13], Godefroot *et al.*[2], Grob *et al.*[14], Traitler and Prévôt[15] and Brechbühler[16]. The fine structures are due to resolution according to the number of unsaturated fatty acids (NUFA) in the triglyceride molecules[17]. For fats and oils containing significant amounts of short-chain fatty acids, resolution is also due to different combinations of chain lengths for a particular carbon number[14]. However, no resolution is obtained for positional isomers (PPO and POP are not separated), or for differences in unsaturation within the unsaturated fatty acids (POP and PLP are not separated). Figs. 2–9 show refined separations on the 15-m column of milk fat, palm kernel oil, coffee oil, palm oil midfraction, mowrah oil, peanut oil, mango fat and interesterified cocoa butter.

Concerning the quantitative aspects of the injection device, the triglycerides of cocoa butter were analysed ten times and the mean peak areas, the standard deviations (S.D.) and the relative standard deviations (coefficients of variation, C.V.) were calculated (Table I).

The coefficients of variation for the main components, C_{50}, C_{52} and C_{54}, are 0.19, 0.12 and 0.20, respectively, illustrating the value of the injection device. This particular analysis was carried out at an oven temperature of 330°C during the injection, reducing the analysis time on the 15-m column to 7 min.

With the use of the injector described, an analysis can be performed every 8–9 min, whereas with a conventional on-column injector it takes at least 30 min.

The mean peak areas, and the S.D. and C.V. values for the analysis of a fatty



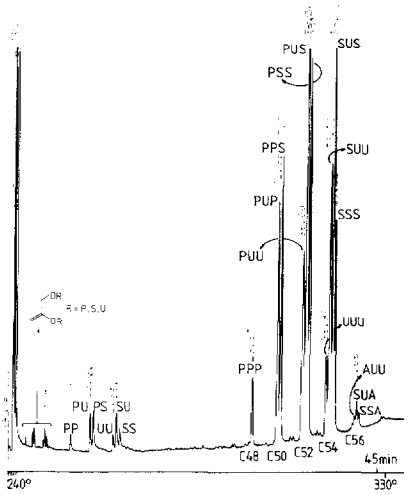


Fig. 8. Analysis of mango fat by capillary GC. Sample, 0.05 μl of 0.1 % mango fat in isooctane; column, as in Fig. 2; oven temperature, 240°C, 1 min isothermal, 2°C/min to 330°C.

Fig. 9. Analysis of cocoa butter by capillary GC. Sample, 0.05 μl of 0.1 % interesterified cocoa butter; conditions as in Fig. 8.

acid methyl ester (FAME) mixture are given in Table II. The oven temperature was held at 140°C during the injection. As can be deduced from Table II, the CV values found in this instance are also very low.

The analysis of very volatile compounds can also be performed with the injection device. The injector then remains in the oven[18].

Returning to the triglyceride analysis, in a previous paper[17] we described the ozonolysis of triglyceride mixtures, facilitating their structural elucidation. More examples illustrating the potential of this method are described here. All ozonized samples were introduced with the movable on-column injector. The analysis time for the examples shown did not exceed 7 min.

The principle of ozonization can be summarized as follows. All double bonds of the triglycerides are attacked by ozone, and aldehydes are formed after reduction with triphenyl phosphine. The molecular weight of the ozonized triglycerides is a

TABLE I

PEAK AREAS FOR 10 INJECTIONS OF COCOA BUTTER

Carbon No.	*Mean peak area (%)*	*S.D. (%)*	*C.V. (%)*
48	0.399	0.018	4.5
50	17.627	0.034	0.19
51	0.406	0.016	3.89
52	45.177	0.056	0.12
53	0.460	0.023	4.91
54	33.551	0.067	0.20
56	1.955	0.030	1.51
58	0.263	0.063	24.05

TABLE II

PEAK AREAS FOR 10 INJECTIONS OF FAME

FAME Carbon No.	*Mean peak area (%)*	*S.D. (%)*	*C.V. (%)*
12	0.638	0.0193	3.02
14	0.991	0.0218	2.2
16	24.699	0.0534	0.22
16:1	0.665	0.0201	3.02
17	0.072	0.0062	8.6
18	33.519	0.0703	0.21
18:1	32.934	0.0712	0.216
18:2	3.189	0.009	0.28
20	1.114	0.0097	0.87
18:3	1.110	0.0082	0.74
22	0.505	0.0189	3.75

function of the number of unsaturated fatty acids in the triglyceride and of the location of the first double bond (starting from the ester function) of the unsaturated fatty acids.

Most natural fatty acids have their first double bond at the 9-position. For example, olive oil consists mainly of triglycerides of the type UUU, and ozonization leads to the $\Psi(9)$ $\Psi(9)$ $\Psi(9)$ major peak (Fig. 10).

For lard (Fig. 11) the major peaks proved to be P$\Psi\Psi$ and PSΨ, both derived

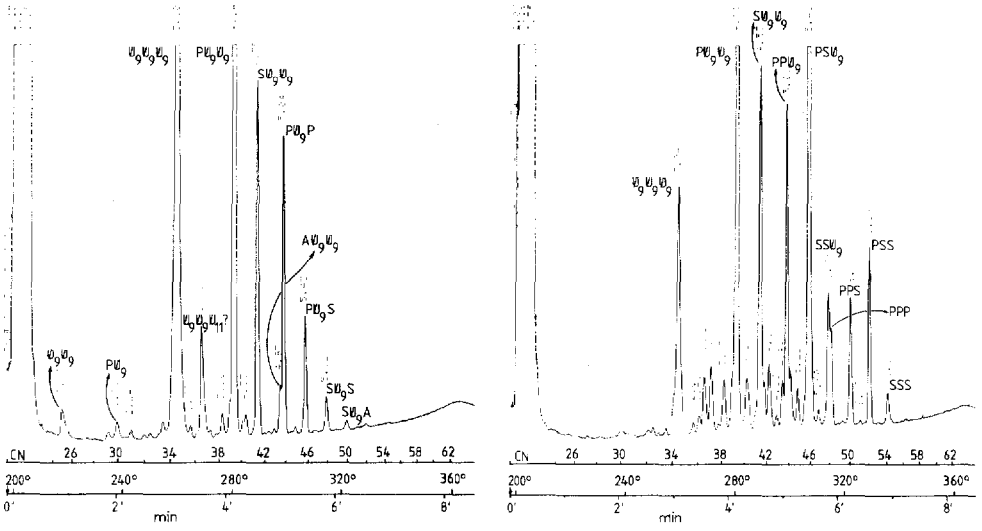


Fig. 10. Analysis of olive oil by capillary GC. Sample, 0.15 μl of ozonized olive oil in isooctane; column, 5 m × 0.3 mm I.D. HTS-OV-1; oven temperature, 200°C, 0.1 min isothermal, 20°C/min to 360°C, 0.9 min isothermal; carrier gas, hydrogen, 0.45 bar.

Fig. 11. Analysis of lard by capillary GC. Sample, 0.15 μl of ozonized lard in isooctane; conditions as in Fig. 10.

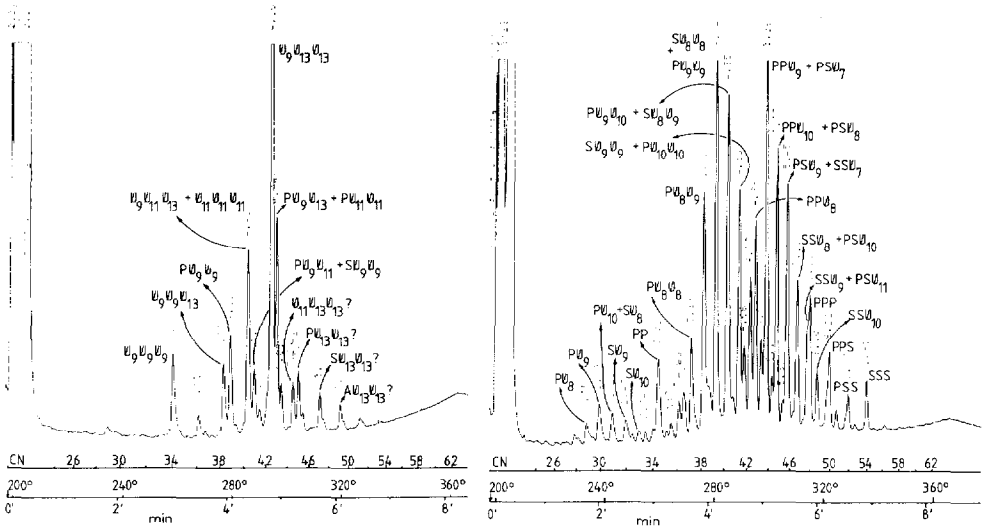


Fig. 12. Analysis of rapeseed oil by capillary GC. Sample, 0.15 μl of ozonized rapeseed oil (high erucic acid content) in isooctane; conditions as in Fig. 10.

Fig. 13. Analysis of palm oil by capillary GC. Sample, 0.15 μl of ozonized partially hydrogenated palm oil fraction in isooctane; conditions as in Fig. 10.

from C_{52}. The quantitative analysis of the trisaturated triglycerides PPP, PPS, PSS and SSS is simplified by ozonization.

Erucic acid is a monounsaturated fatty acid, having the double bond located at the 13-position. High-erucic acid rapeseed oil, consisting mainly of oleodierucate, OErEr (C_{62}), which gives rise to the triglyceride peak, $\Psi(9)\Psi(13)\ \Psi(13)$ (C_{35}) (Fig. 12), clearly with a higher theoretical carbon number than olive oil, $\Psi(9)\ \Psi(9)\ \Psi(9)$ (C_{27}), and most common unsaturated vegetable oils.

Perhaps the most promising application of the triglyceride analysis of ozonized fats is the study of fat hydrogenation. During contact of the unsaturated triglycerides with a hydrogenation catalyst, the double bonds will be dislocated from their original positions. This dislocation can occur in both directions along the carbon chain and we can expect a random distribution of the double bonds around their original positions. This random distribution is recognized in the pattern of the ozonized triglycerides. The chromatogram of an ozonized partially hydrogenated palm oil fraction (Fig. 13) clearly illustrates this phenomenon.

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