

## Improved conditions for gas-liquid chromatography of triglycerides

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**SUMMARY** Improved conditions for gas-liquid chromatography of triglycerides are reported. Triglycerides of bovine colostrum and rapeseed oil have been analyzed under these conditions.

**KEY WORDS** triglycerides · gas-liquid chromatography · column preparation · on-column injection · dual-column operation · bovine colostrum · rapeseed oil

THE FIRST adequate separations of natural triglyceride mixtures by GLC were reported more than 4 years ago (1). Under the conditions described, however, entirely satisfactory resolutions can be obtained only infrequently and attempts to reproduce them have given rise to disappointments in a number of laboratories. Recently we have been able to prepare excellent columns for the GLC of triglycerides with great regularity and have noted that the critical factor is the efficiency with which the sample is delivered to the packing. This communication describes the improved columns and operating conditions.

*Instruments.* Two gas chromatographs were used. The single column instrument was an Aerograph Hy-Fi, Model 600 D (Wilkins Instrument and Research, Inc., Walnut Creek, Calif.). It was used together with a Power Proportioning Linear Temperature Programmer (Model 240, F & M Scientific Corporation, Avondale, Pa.), and an Electronik 15 1-mv recorder (Minneapolis-Honeywell Regulator Co., Philadelphia, Pa.). The dual column instrument was an Aerograph 204-1B Dual Channel Gas Chromatograph with dual flame detectors, a dual channel electrometer, and a linear temperature programmer. It was equipped with the same recorder.

*Columns.* Columns consisted of stainless steel tubes, 50 cm × 3 mm o.d., 0.5 mm wall, which were equipped with Swagelok fittings in such a manner as to allow the insertion of the injector end of the column into the flash evaporator up to 6 mm from the silicone septum (see Fig. 1). The tube was consecutively washed with about 100 ml each of methanol, diethyl ether, and acetone by sucking the solvents through the tube by means of a water pump. The tube was briefly heated in an oven to drive off the solvents and any traces of moisture. After

Abbreviations: GLC, gas-liquid chromatography; C<sub>24</sub>, C<sub>66</sub> triglycerides, etc., triglycerides with a total number of fatty acid carbons of 24, 66, etc.

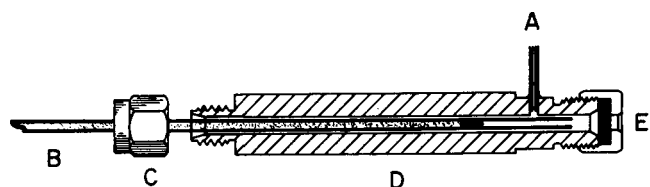


FIG. 1. Positioning of column in the injector assembly. *A*, carrier gas inlet; *B*, packed column; *C*, Swagelok nut (front and back ferrules not shown); *D*, injector barrel; *E*, injector nut and silicone septum.

the tube had cooled to room temperature it was siliconized with a 5% (v/v) solution of hexamethyldisilazane in chloroform (50 ml), the solution being gently sucked through the tube with the help of the pump. The tube was heated for about 15 min at 100°C, cooled to room temperature, and packed immediately.

**Packings.** Several commercially available and laboratory prepared packings were used. A column packing containing 3% (w/w) JXR (dimethylpolysiloxane gum) on silanized Gas-Chrom Q, 100–120 mesh, was supplied by Applied Science Laboratories Inc., State College, Pa. Another packing containing 5% (w/w) SE-30 (General Electric silicone gum) on acid washed Chromosorb W, 60–80 mesh, was obtained from Wilkens Instrument and Research, Inc. The laboratory packings containing 1–2% (w/w) liquid phase were prepared by solution coating (2) of dimethyldichlorosilane-treated Chromosorb W, 70–80 mesh (Wilkens) with a 1% (w/v) toluene solution of JXR (Applied Science) or SE-30 (Wilkens).

**Filling the Columns.** Before the steel tube was filled, its outlet was closed with a compact plug of siliconized glass wool (Applied Science Laboratories Inc.), extending about 6 mm from the end of the tube into the column interior. The plugged end was attached to a water pump and the column filled under suction with the help of a small funnel attached to the column inlet by Tygon tubing. During packing the tube was mechanically vibrated (Vibro-Graver, Burgess Vibrocrafters, Inc., Grayslake, Ill.). The column was firmly packed to about 35 mm from the inlet and a small siliconized glass wool plug was pushed down the tube to rest against the packing. The amount of packing accommodated by the column was quite reproducible: 0.8–0.9 g per 45 cm of packed length. When the dual column instrument was to be used, two columns were packed simultaneously under closely similar conditions.

**Conditioning of Columns.** Columns were conditioned as follows. The nitrogen flow rate was adjusted to about 100 ml/min at room temperature, and the temperatures of the oven and of the injector block were increased to 350°C and kept there for 6–8 hr. The injector was then allowed to cool to 200–250°C, when the oven heater was turned off, the lid of the oven opened, and the column cooled to room temperature. The column outlet was con-

nected to the detector; the nitrogen, hydrogen, and air flows were adjusted to the desired rates and the whole system was checked for leaks. The temperatures of the detector, oven, and injector were adjusted (in that order) to the working or starting levels. The column was further conditioned by injecting small amounts (1  $\mu$ l at a time) of petroleum ether or chloroform and programming the oven temperature to rise to 350°C at 2 or more degrees per minute; the base line elevation was noted. In the dual column instrument each column was equilibrated separately, and the relative flow rates of the carrier gas in the two columns were adjusted at the maximum operating temperature until comparable bleeds of stationary phase were obtained, with no noticeable base line drift. Usually several solvent injections followed by temperature programming of the column were necessary before a stable base line could be obtained.

The equilibrated columns were checked with a mixture of equal parts (by weight) of trilaurin, trimyristin, tripalmitin, and tristearin (Applied Science Laboratories Inc.). If the recorded areas corresponded to the original weight proportions of the test mixture, it was concluded that the column gave complete recoveries of all the saturated triglycerides. The recoveries of trilaurin, trimyristin, and tripalmitin were usually quantitative (100% with respect to trilaurin) with these columns after the first conditioning. If the area response was low for tristearin, the conditioning of the column at 350°C was repeated until at least 90% of the theoretical recovery was obtained. The recovery of the unsaturated triglycerides was checked by substituting triolein (Applied Science Laboratories Inc.) or trilinolein (Procter & Gamble Mfg. Co., Cincinnati, Ohio) for tristearin in the test mixture. Under comparable conditions the recovery of triolein was up to 5% higher than that of tristearin, while that of the crude trilinolein was 25–50% lower. As the columns aged, the proportional recoveries of the long-chain triglycerides increased; this necessitated frequent determination of correction factors for quantitative work.

**Injection of Samples.** All samples were injected directly into the packing by means of a 10  $\mu$ l Hamilton syringe (Hamilton Company, Whittier, Calif.) equipped with a 2 inch needle, which could be felt to just reach into the packing during the injection (CAUTION: do not crush the support!). One microliter of a 1% solution in chloroform or petroleum ether (bp 60–70°C) was injected.

**Separations of Natural Triglyceride Mixtures.** Fig. 2 shows the elution pattern recorded for the triglycerides of bovine colostrum, which contains short-chain and long-chain triglycerides. The separation is complete within 40 min; there is nearly complete return to the base line between each pair of adjacent peaks. Triglyceride mixtures which contain no odd-numbered

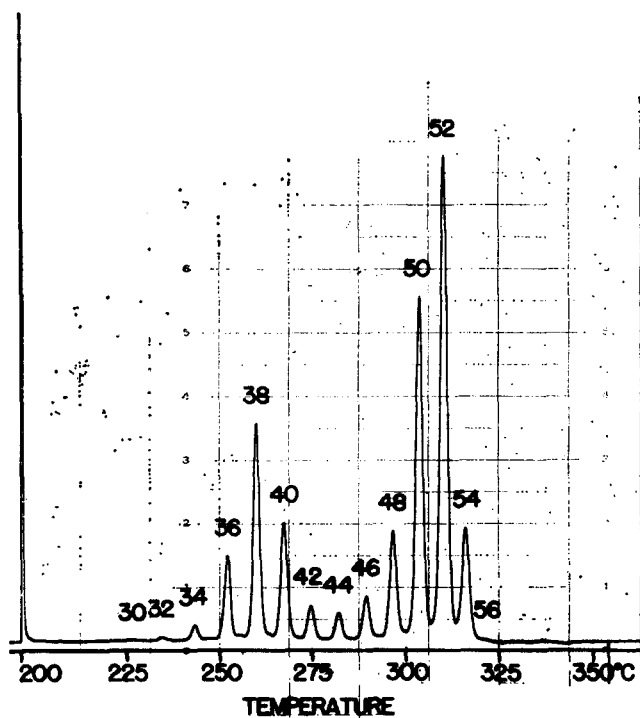


FIG. 2. GLC of triglycerides of cow's colostrum. Peaks identified by the total number of carbon atoms in the fatty acid moieties.

*Instrument:* Aerograph 204-1B (dual column). *Column:* stainless steel tube, 50 cm  $\times$  3 mm o.d., packed with 3% (w/w) JXR on Gas-Chrom Q (100-120 mesh) and assembled as shown in Fig. 1. *Carrier gas flow rate:* 120 ml/min of  $N_2$  at room temperature, 75% of maximum flow. The injector was at 280°C, the detector at 325°C. *Sample:* 1  $\mu$ l of 1% (w/v) triglyceride solution in chloroform. *Attenuation:* 1  $\times$  2. *Temperature program as shown. Chart speed:* 6 min/inch.

fatty acids gave complete return to the base line between peaks. Some 20 columns have been packed in the manner indicated, with two different batches of JXR packings, and some 200 comparable analyses have been completed, both of standard triglycerides and the triglyceride mixtures present in bovine colostrum.

The conditions given in Fig. 2 allowed, however, complete recoveries of only those triglyceride mixtures for which the total fatty acid carbon number did not exceed  $C_{56}$ . For higher molecular weight glycerides, higher injector and column temperatures are necessary. These separations are best performed in a dual column instrument in which the greatly increased "bleeding" of the liquid phase at the elevated temperatures is compensated for, so that high sensitivity settings, which are more efficient for the analysis of the larger molecules, can be used. An example of a separation under these conditions is shown in Fig. 3. Triglycerides with 50-62 fatty acid carbon atoms were completely separated and recovered in high yield within 10 min of the injection. Although the recoveries of triglycerides higher than  $C_{56}$  were not studied in detail, it was calculated (3) on the basis of the fatty acid composition of the rapeseed oil (Canada

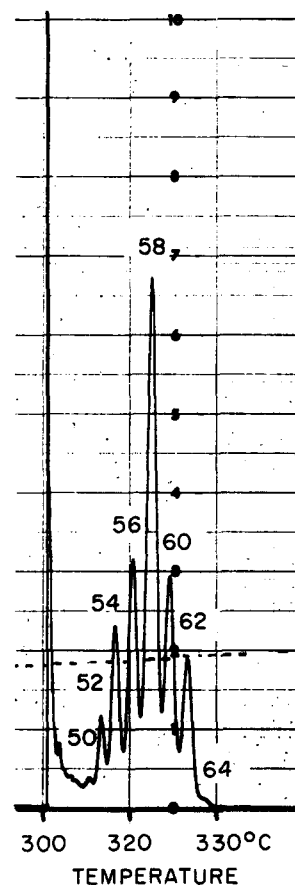


FIG. 3. GLC of triglycerides of rapeseed oil (at higher temperature). *Sample:* 1  $\mu$ l of 0.5% (w/v) triglyceride solution in chloroform. *Attenuation:* 1  $\times$  8. The injector was at 325°C, the detector at 350°C. *Temperature program as shown. Other conditions as in Fig. 2.*

Packers Ltd., Toronto, Canada) and the proportions of the glyceride peak areas observed on GLC that over 80% of the theoretical yield of the fatty acid carbon was recovered.

For a continuous resolution of triglycerides with fatty acids totaling 24-66 carbon atoms it was necessary to program also the temperature of the "injector barrel," preferably at a somewhat higher rate than that selected for the column, otherwise the recoveries of the longer-chain triglycerides were greatly reduced. Under the described experimental conditions, the injector temperature should not exceed the column temperature by more than 50-75°C, otherwise substantial amounts of the liquid phase will be moving down the column and will appear as false peaks in the detector.

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