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IMPROVED SEPARATION OF TRIGLYCERIDES AT LOW TEMPERATURES BY REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

A high-performance liquid chromatographic technique for the separation of triglycerides in natural mixtures is described. The influence of column temperature on separation is investigated, and a linear relationship between log k' and 1/T is demonstrated. Operating this reversed-phase technique with a LiChrosorb RP-18 packing at 14.5°C, separation of triglycerides with the same equivalent carbon numbers (ECN), but different degrees of saturation, is obtained.

The composition of an eluent which permits separation of saturated triglycerides with carbon numbers as high as 54 (*e.g.*, tristearin) is given.

INTRODUCTION

Interest in separating and analysing mixtures of natural fats is still growing, and high-performance liquid chromatography (HPLC) provides a powerful tool for the rapid analysis of fats. The use of argentation HPLC with silica particles loaded with silver ions permits separation of the positional isomers of triglycerides[1], but this normal-phase HPLC is difficult to run routinely. The literature contains many examples of triglyceride separations with HPLC in the reversed-phase mode[2–5]. All these references deal with separation at room temperature on C_{18} columns, and most of them lead to separation according to the equivalent carbon number (ECN). However, Herslöf *et al.*[3] and Vonach and Schomburg[5] have demonstrated the separation of the ECN 48 triglycerides containing oleic and palmitic acid, but so far no author has demonstrated elution of tristearin in reversed-phase HPLC. Plattner *et al.*[2] state that this triglyceride precipitates in the injector when their solvent system is used. Recently, Gilpin and Sisco[6] have investigated the effect of temperature on the retention times of some aromatic compounds; they found a linear relationship in the reversed-phase mode between ln k' and $1/T$ (where k' is the capacity ratio and T is the column temperature).

This paper demonstrates an improved separation of triglycerides from natural mixtures by reversed-phase HPLC at sub-ambient temperature and gives the composition of a solvent system that permits elution and separation of unsaturated and of saturated triglycerides containing fatty acids with chain lengths up to C_{18}.

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EXPERIMENTAL CONDITIONS

The HPLC was carried out using the following equipment: a Waters Assoc. pump (Model M 6000) delivering 1.5 ml/min, with a 25 cm × 4.6 mm stainless-steel column (Knauer) packed with LiChrosorb RP-18 (5 μm) (Merck, Darmstadt, G.F.R.). The column was packed in the laboratory in the upwards-slurry technique with the Waters pump.

The samples were injected as chloroform solutions by means of a U6K universal-loop injector (Waters), and a Waters differential refractometer (Model 401) was used as detector. The column was thermostatically controlled at sub-ambient temperature (routinely 14.5 ± 0.1°C) by means of Hetofrig cooling system (type 03PF623CB11).

The following solvent system was used: acetonitrile (Merck; art. 3)–tetrahydrofuran (Merck; art. 9731)–*n*-hexane (Merck; art. 4391) (224:123.2:39.6, w/w/w).

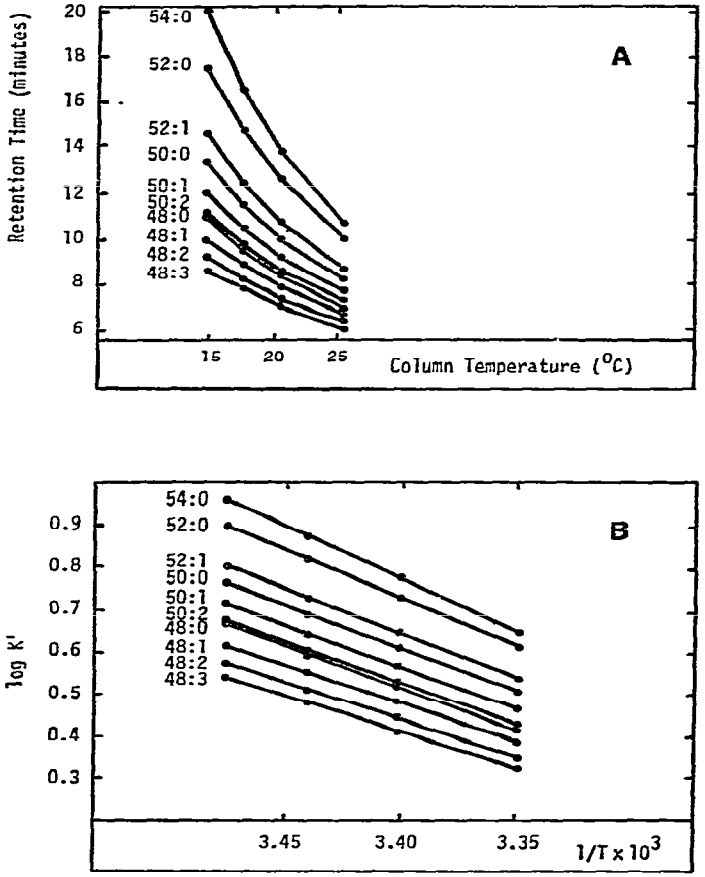

Fig. 1. A, Plots of retention time *vs.* column temperature for various triglycerides from vegetable oils. B, Plots of log *k'* *vs.* $1/T$ for the same triglycerides as in A. Eluent: acetonitrile–tetrahydrofuran–*n*-hexane; flow-rate 1.5 ml/min. The number at the end of each curve indicates the equivalent carbon number (ECN) of the fatty acids in the triglycerides as well as the number of double bonds, *e.g.*, 48:3 (ECN 48; 3 double bonds).

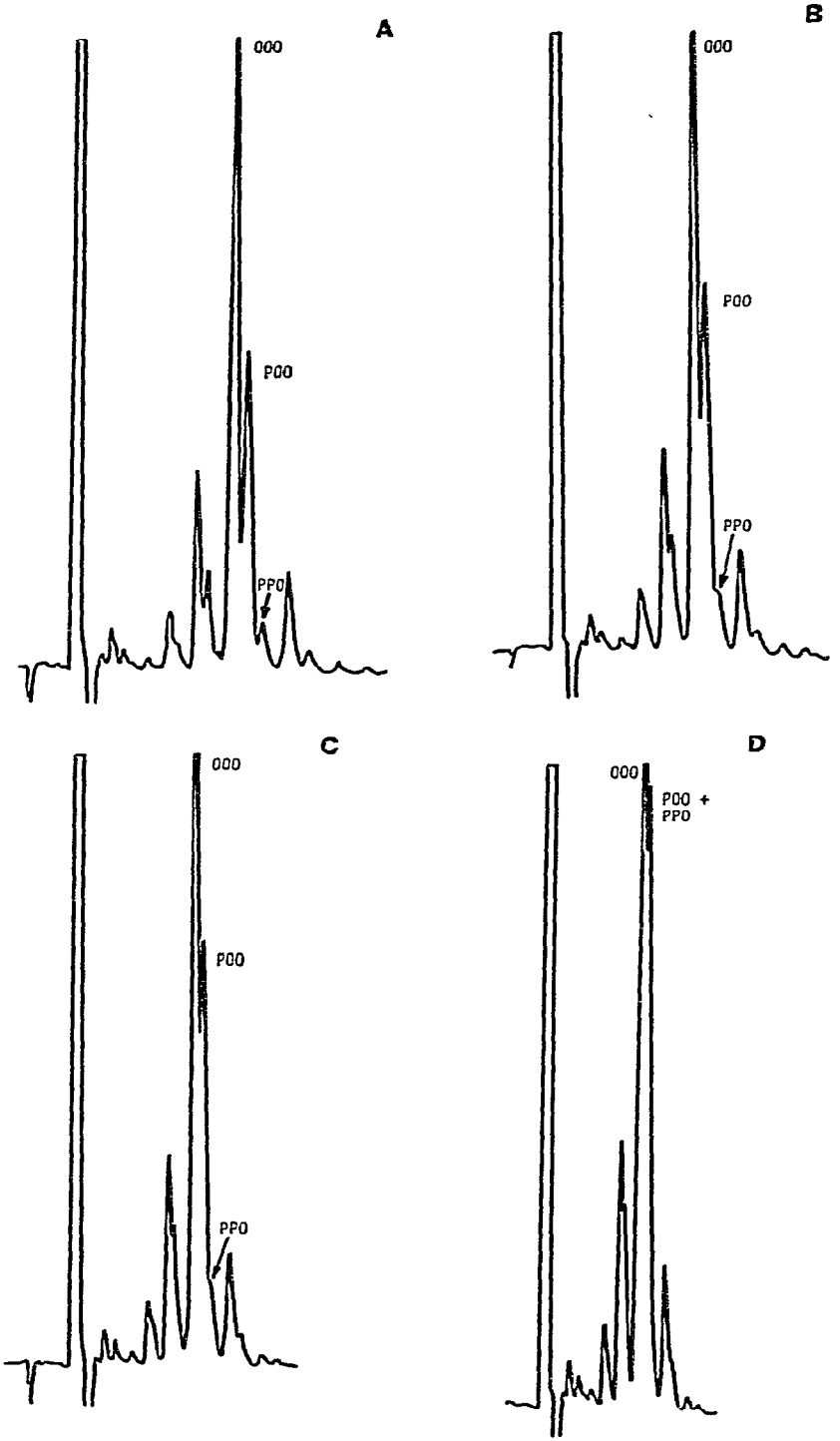

Fig. 2. Separation of triglycerides in olive oil at different temperatures. A, 14.5°C; B, 17.5°C; C, 20.5°C; D, 25.5°C. Chromatographic conditions as in Fig. 1.

It was necessary to weigh the components, as even small variations could cause shifts in the retention times. The samples used for investigating the influence of column temperature on the separation of triglycerides were olive oil, coconut oil and selected triglycerides, such as POS, POP, SOS, SSS and other standards (P = palmitic acid; O = oleic acid, and S = stearic acid); 5 μl of a 10% solution of the oil in chloroform and 5 μl of 4% standard triglyceride solution in chloroform were used.

RESULTS AND DISCUSSION

The purpose of this work was to establish an isocratic reversed-phase method for separating triglycerides in natural vegetable fats and oils, such as olive oil and coconut oil, and which permits elution of tristearin.

The influence of the column temperature on the separation was investigated by making several runs at four temperatures (14.5, 15.5, 20.5 and 25.5°C).

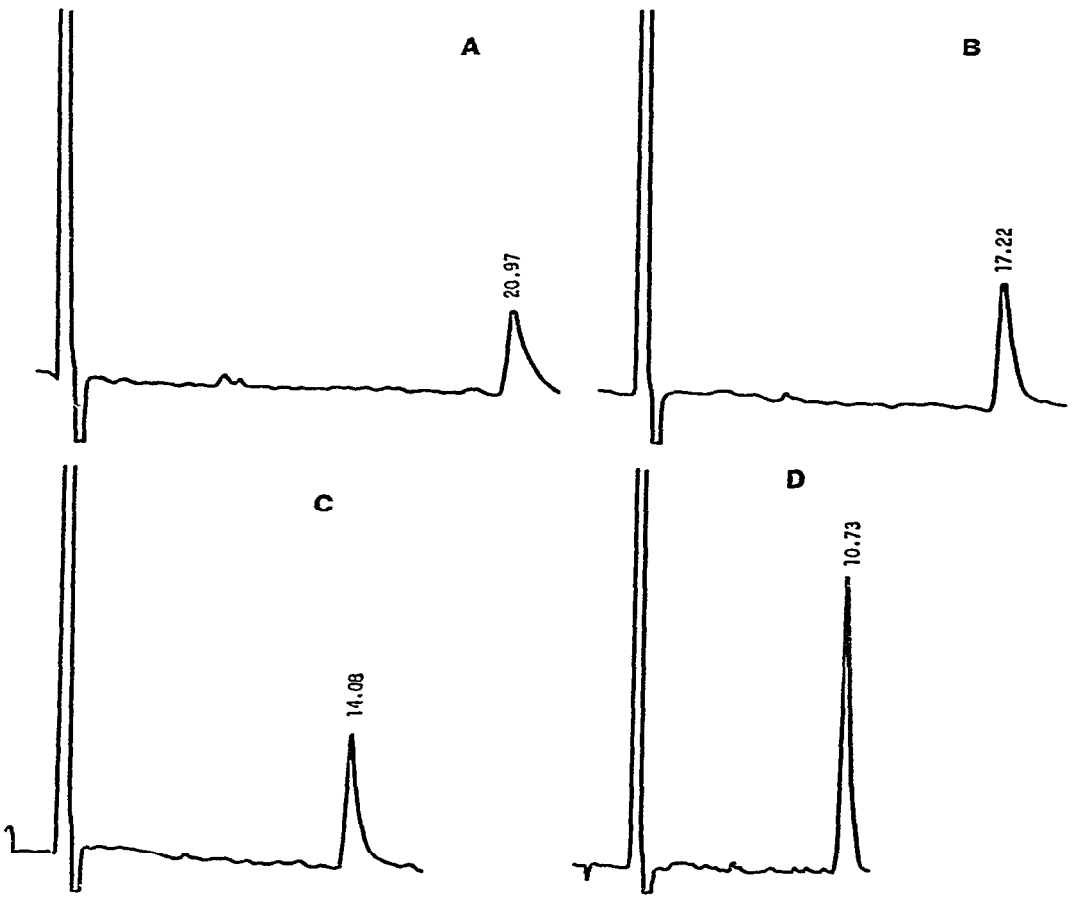

Fig. 3. Elution of tristearin at different temperatures. A, 14.5°C; B, 17.5°C; C, 20.5°C; D, 25.5°C. The number of the above the peak indicates the retention time (in minutes) at the temperature indicated. Chromatographic conditions as in Fig. 1.

From Fig. 1A it is obvious that the best separation is obtained at *ca.* 14.5°C, as the distance between the lines drawn for each triglyceride is here at its maximum (see also Fig. 2). At lower temperatures, the solubility, especially of tristearin (ECN 54:0), in the solvent falls drastically. The number at the end of each curve indicates the equivalent carbon number (ECN) of the fatty acids in the triglyceride as well as the number of double bonds within the triglyceride, *e.g.*, triolein is 48:3 (ECN 48; 3 double bonds).

Changes in column temperature have the greatest influence on retention time the longer and the more saturated the fatty acids in the triglycerides are. This effect is due to the more hydrophobic character of the long-chain saturated fatty acids and thereby a greater temperature-dependent affinity to the stationary phase.

Fig. 1B shows the relation between log k' and $1/T$ for the same triglycerides as in Fig. 1A. This relationship is linear and in good agreement with the results reported for aromatic compounds by Gilpin and Sisco[6]. None of the investigated triglycerides shows non-linear behaviour within the temperature range examined.

Fig. 2 shows four chromatograms of an olive oil separation at the four temperatures shown in Fig. 1; it demonstrates the chromatograms obtainable at these temperatures, and confirms that the best separation is obtained at *ca.* 14.5°C.

In Fig. 3, the effect of temperature on the elution of tristearin is demonstrated. At 25.5°C, tristearin is eluted as a sharp peak, virtually without tailing, but at lower temperatures it is eluted as a broader and skewed peak, the width increasing as the temperature decreases. This broadening effect is due to the low solubility of tristearin in the eluent and to its great affinity to the stationary phase, especially at low temperatures. Using a stronger eluent to overcome the boradening effect results in poorer resolution of the component preceding tristearin, so the eluent used is considered to offer a fairly good compromise.

CONCLUSION

Reversed-phase HPLC at low temperatures makes it possible to separate triglycerides, not only according to their ECN, but also according to their degree of saturation. The solvent system used permits elution of tristearin as a sharp peak at room temperature within 12 min, and within 22 min as a broader peak at 14.5°C.

REFERENCES

1 E. C. Smith, A. D. Jones and E. W. Hammond, *J. Chromatogr.*, 188 (1980) 205–212.
2 R. D. Plattner, G. F. Spencer and R. Kleiman, *J. Amer. Oil Chem. Soc.*, 54 (1977) 511–515.
3 B. Herslöf, O. Podlaha and B. Töregaard, *J. Amer. Oil Chem. Soc.*, 56 (1979) 864–866.
4 D. W. Lehrian and P. G. Keeney, *J. Amer. Oil Chem. Soc.*, 57 (1980) 66–69.
5 B. Vonach and G. Schomburg, *J. Chromatogr.*, 149 (1978) 417–430.
6 R. K. Gilpin and W. R. Sisco, *J. Chromatogr.*, 194 (1980) 285–295.