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Short Communication

Permanganate-impregnated packed capillary columns for group separation of triacylglycerols using supercritical media as mobile phases

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ABSTRACT

Capillary columns packed with a silica-based anion exchanger were treated *in situ* with a solution of potassium permanganate. These columns showed highly reproducible retention times when used under supercritical conditions. Column temperatures up to 140°C could be applied. This type of column was successfully used for the group separation of triacylglycerols from vegetable oils according to their degree of unsaturation.

INTRODUCTION

Transition metal complexes provide high selectivities when used as stationary phases in chromatography [1], and a wide variety of metal acceptors have been shown to form complexes with olefins [2]. The selectivity obtained with Ag^+ was found to be particularly useful, and silver-containing stationary phases are now being extensively used. Christie [3] showed that very stable argentation columns for liquid chromatography were obtained when a silica-based cation modifier was used as a support for the silver ions. We have recently shown that capillary columns packed with a silver-impregnated cation exchanger are remarkably stable under supercritical fluid chromatographic (SFC) conditions [4,5]. These results encouraged us to evaluate the interactions in SFC with olefins of some other transition metal complexes.

Metal atoms of oxides are coordinated to silanol surface groups [6] and silica-carrying metal oxides has been used as catalysts. Metals such as Cr [7], Mn [8] and V [9] have been used. In this work, the separation of triacylglycerols on permanganate-impregnated columns in SFC was investigated. In the early days of gas chromatography (GC), manganese stearate was tried as a stationary phase for the separation of amines [10]. In addition, chelates containing manganese (II) have been used in GC [17]. Manganese is a weak acceptor [11]; however, the aim of this work was separation into groups rather than separation of molecular species of triacylglycerols.

EXPERIMENTAL

The chromatographic system consisted of a Lee Scientific 600 Series SFC system and an Isco μLC-10 variable-wavelength absorbance detector. Detection was performed at 210 nm on a short

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length of 250-μm I.D. fused-silica tubing according to the method of Fields *et al.* [12]. The width of the slit on the detection capillary was about 1 mm. Fused-silica capillary tubing (Polymicro Technologies, Phoenix, AZ, USA) of 11 μm I.D. was used as a restrictor in lengths of 20–25 mm.

Columns were prepared from fused-silica capillary tubing, 290 or 250 mm × 0.25 mm I.D. and 0.43 mm O.D. (Polymicro Technologies). The columns were packed with Nucleosil 5 SB (Macherey–Nagel, Düren, Germany) or Supersphere Si 60, 4 μm (Merck, Darmstadt, Germany). Slurry packing was excecuted as described previously [4]. After packing, the columns were washed with methanol, 400 μl of distilled water, 400 μl of 0.2 *M* potassium permanganate solution and 400 μl of distilled water. Finally, the columns were dried by flushing with carbon dioxide at 115°C and 275 atm.

The mobile phase consisted of carbon dioxide–acetonitrile–isopropanol, different contents of modifier being used for the different column types and the content of isopropanol being *ca.* 10% of that of acetonitrile. SFC-grade carbon dioxide (Scott Specialty Gases, Plumsteadville, PA, USA) was used. The mobile phase mixture was prepared in the SFC pump as described previously [13]. The mobile phase velocity was 3.5 mm/s.

Chromatographically purified olive oil, corn oil, soyabean oil and linseed oil were purchased from Larodan Fine Chemicals (Malmö, Sweden) and borage oil was obtained from LipidTeknik (Stockholm, Sweden). The solutes were dissolved in high-performance liquid chromatographic (HPLC)-grade pentane at concentrations of 30 mg/ml. Injection was performed with a splitting ratio of 1:1 and a timed split of 0.2 s.

RESULTS AND DISCUSSION

Excellent reproducibility of retention times was observed under the applied conditions. Further, the columns could be used at temperatures up to 140°C without deterioration of the performance.

The separation of some different vegetable oils is demonstrated in Figs. 1–5. As shown in Figs. 1–4, good group separation of the triacylglycerols according to the number of unsaturations was obtained. Fig. 5 shows the separation of a highly com-

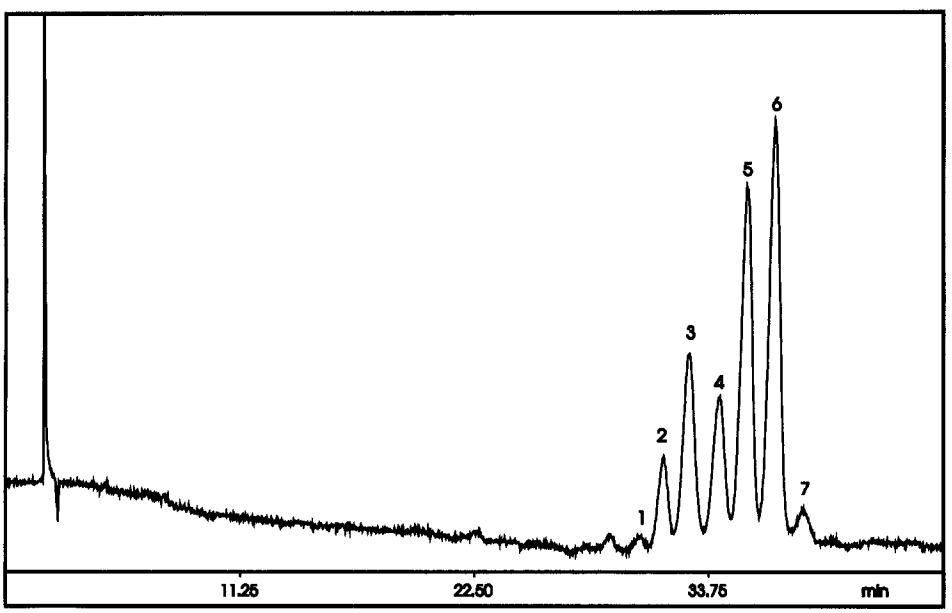

Fig. 1. Supercritical fluid chromatogram of corn oil. Capillary column (330 mm × 0.25 mm I.D.) packed with 5-μm anion exchanger and modified *in situ* with $KMnO_4$. Injection at 100°C and 275 atm; after 2 min, programmed at −1°C/min to 75°C and 0.5 atm/min to 288 atm. Mobile phase: carbon dioxide–acetonitrile–isopropanol (97.3:2.4:0.3, mol%). UV detection at 210 nm. Peak numbers refer to the number of double bonds.

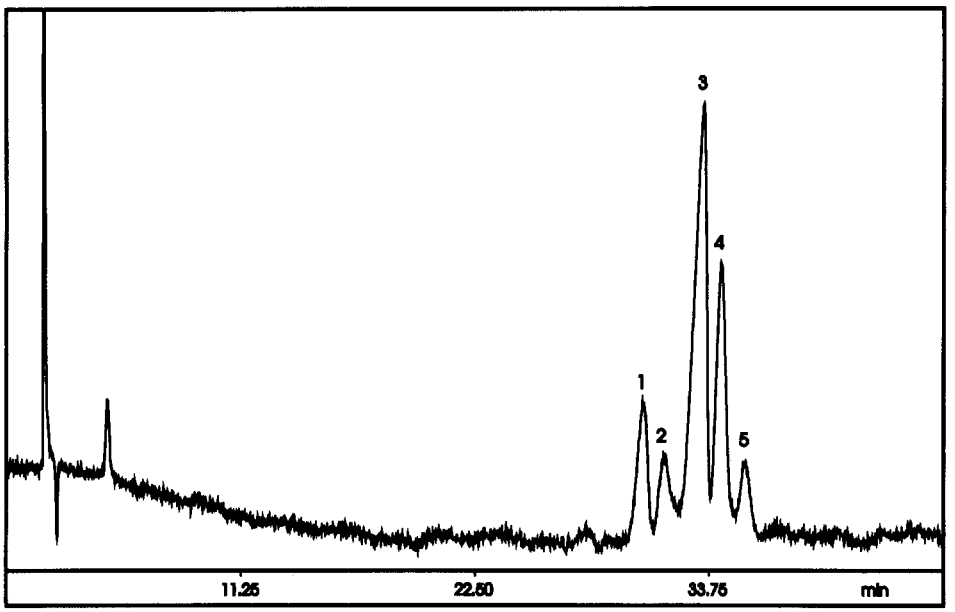

Fig. 2. Supercritical fluid chromatogram of olive oil. Column, conditions and peaks as in Fig. 1.

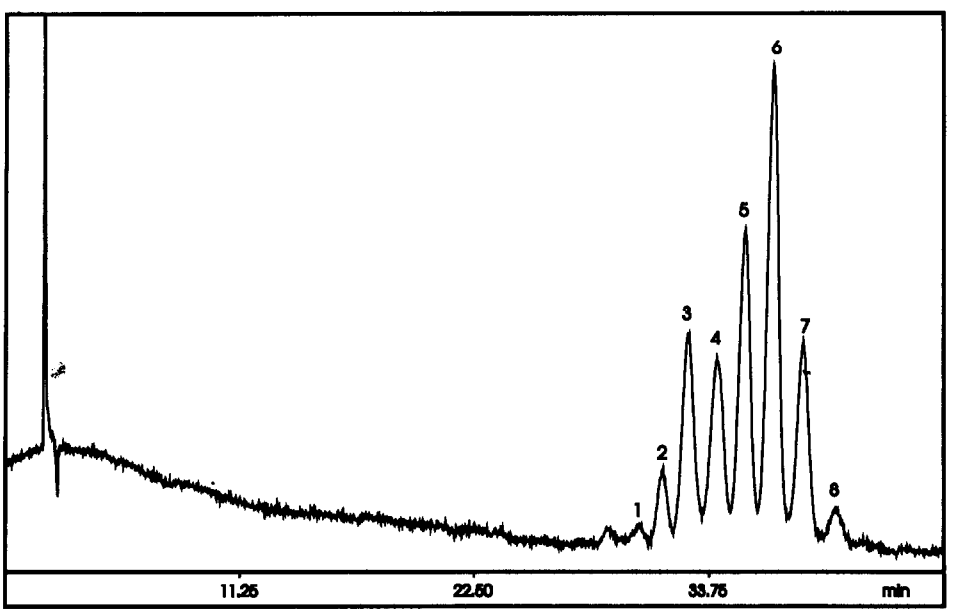

Fig. 3. Supercritical fluid chromatogram of soyabean oil. Column, conditions and peaks as in Fig. 1.

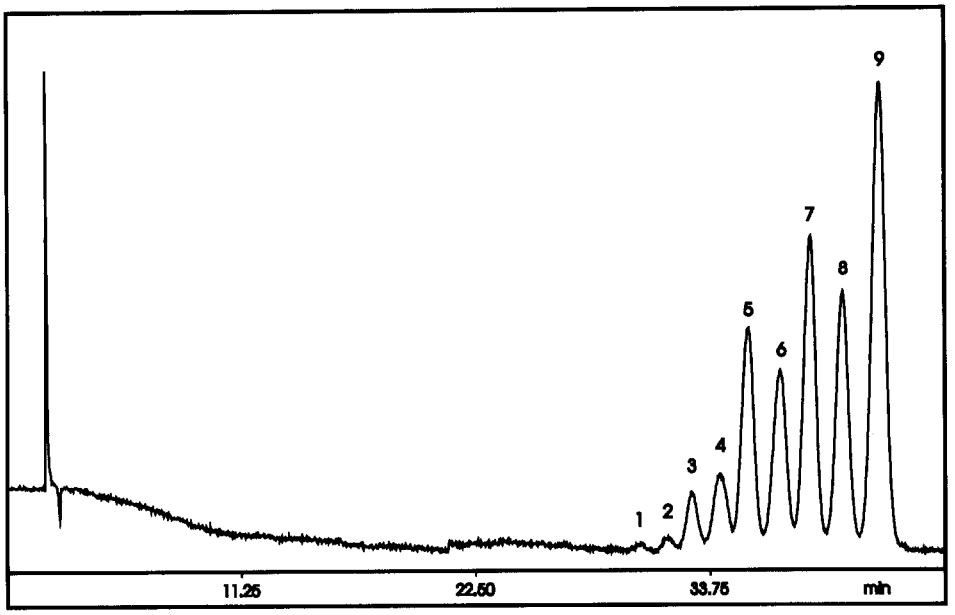

Fig. 4. Supercritical fluid chromatogram of linseed oil. Column, conditions and peaks as in Fig. 1.

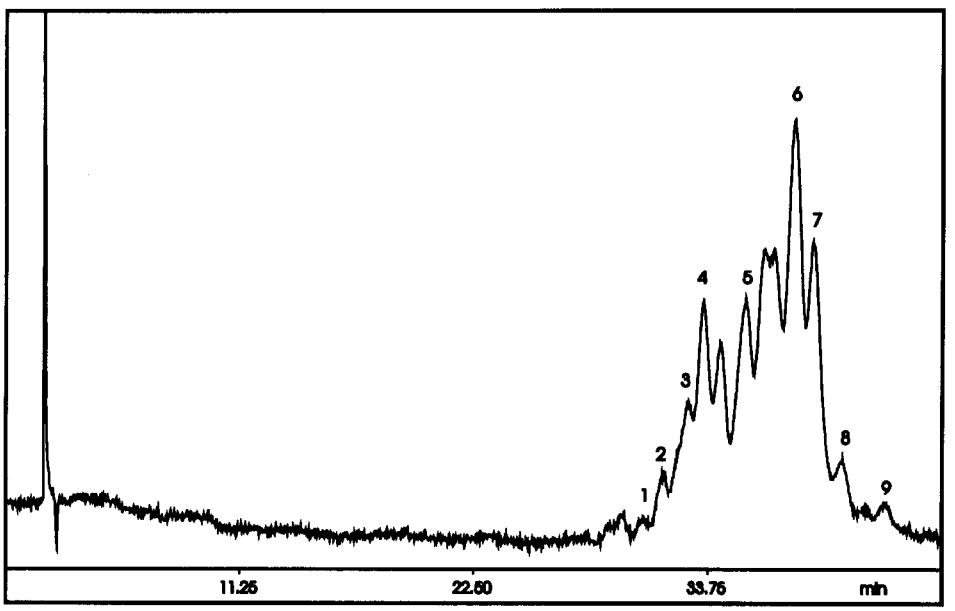

Fig. 5. Supercritical fluid chromatogram of borage oil. Column, conditions and peaks as in Fig. 1.

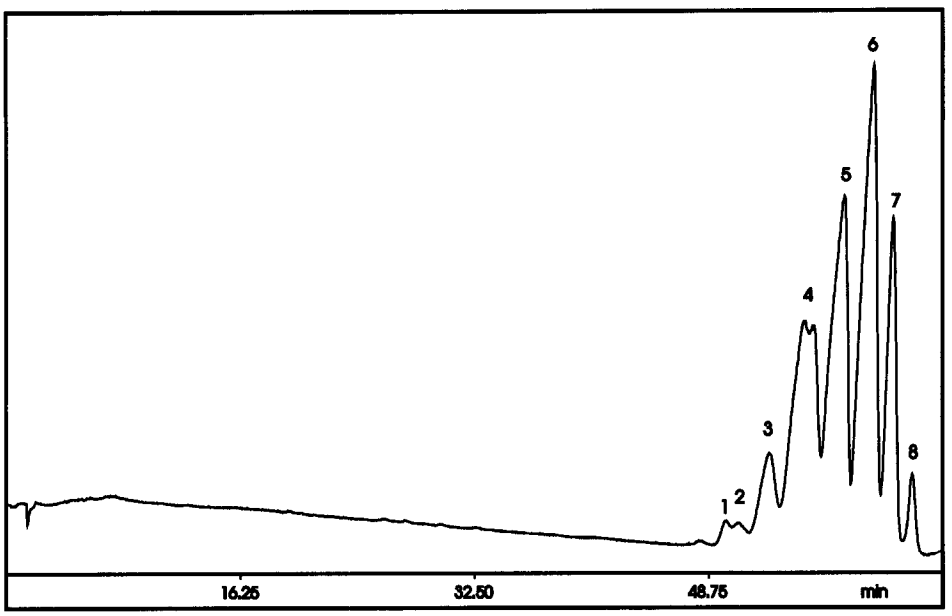

Fig. 6. Supercritical fluid chromatogram of soyabean oil. Capillary column (250 mm × 0.25 mm I.D.) packed with 4-μm silica. Injection at 150°C and 240 atm; after 2 min, programmed at −2°C/min to 70°C and 2 atm/min to 320 atm. Mobile phase: carbon dioxide–acetonitrile–isopropanol (98:1.8:0.2, mol%). UV detection at 210 nm. Peak numbers refer to number of double bonds.

plex oil [14], where some overlap between the different groups was inevitable. A chromatogram of soyabean oil obtained on a capillary packed with silica is shown in Fig. 6. The separation is relatively poor, but it is much more complete than previously reported separations on silica by HPLC [15].

In order to achieve optimum elution of triacylglycerols on permanganate-treated columns, it was necessary to add 2.8% of acetonitrile to the mobile phase. For the elution of the same samples on Ag^+-impregnated columns, 6.5% of acetonitrile was required [5]. The difference depends on two factors. First, the acetonitrile is needed for partial deactivation of the Ag^+ moieties, and second, the acetonitrile serves to deactivate residual surface silanol groups. Triacylglycerols are, however, relatively insensitive towards residual silanol groups, thus giving the poor separation shown in Fig. 6. Fatty acid methyl esters (FAME) are much more sensitive in this respect, and the silanol-deactivating effect of permanganate treatment was recently demonstrated for such compounds [16]. Finally, when using open-tubular columns, triacylglycerols are readily eluted with carbon dioxide alone, the modifier thus not being of crucial importance for the solubility in the mobile phase in this instance.

It has been pointed out that the chromatographic activity of the transition metal complexes cannot be ascribed solely to the central metal atom [1]. The ligands may also play a role through steric factors or inductive factors and, further, they stabilize the oxidation state of the metal. Columns packed with ion exchanger in addition to silica became light brown on permanganate treatment. Obviously, the anion-exchange moieties were degraded by the permanganate. It is not known in which form the manganese occurs on the silica surface, but the presence of manganese was detected by means of atomic absorption spectrometry.

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