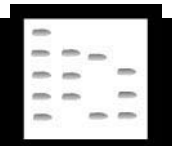
OILS, FATS AND WAXES: SUPERCRITICAL FLUID CHROMATOGRAPHY



F. David, **A. Medvedovici and P. Sandra**,
Research Institute for Chromatography,
Kortrijk, Belgium

Introduction

The analysis and characterization of lipids and waxes is of great importance in the food industry, pharmaceutical and cosmetic industry, in surfactant and detergent technology and in natural product research. Lipid analysts have always been on the forefront of developments in separation sciences and new techniques in chromatography have often been developed for the separation of lipids. The first application of gas chromatography, for instance, was the separation of fatty acids by James and Martin. Since then, all chromatographic techniques, including capillary gas chromatography (CGC), high performance liquid chromatography (HPLC), thin layer chromatography (TLC), capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), capillary electrochromatography (CEC) and supercritical fluid chromatography (SFC) have been applied to the analysis of lipids and also waxes which have similar physico-chemical characteristics.

It is not possible to give a complete overview of the possibilities and limitations of each of these techniques in comparison to supercritical fluid chromatography for these analytes. For a detailed description of the use of supercritical fluids in the analysis of oils, fats and waxes, we refer to a number of recently published books listed in the Further Reading. The possibilities of both capillary column supercritical fluid chromatography (cSFC) and packed column supercritical fluid chromatography (pSFC) in the analysis of lipids and related compounds will be illustrated. The experiences gained in the authors' laboratories over the years are summarized.

The Analytical Challenge

According to Christie, lipids can be defined as 'fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds'. This is a rather broad definition and covers a large number of organic substances. In general, two lipid classes can be distinguished: simple lipids, yielding maximum two primary hydrolysis products after saponification; and complex lipids, yielding three or more hydrolysis products. Triglycerides, natural waxes and sterol esters are examples of simple lipids as they yield fatty acids and glycerol, fatty acids and an aliphatic alcohol or fatty acids and a sterol, respectively, on saponification. Phosphatidylethanolamine (a phospholipid) on the other hand, is a complex lipid as it yields glycerol, phosphoric acid, fatty acids and ethanolamine on saponification.

Lipid mixtures as they occur in natural fats, oils and waxes, are quite complex in their composition. A vegetable oil, for instance, mainly consists of triglycerides, but also contains free fatty acids, monoglycerides, diglycerides, sterols and sterol esters. Within these classes, different combinations of fatty acids are possible. All constituents contribute to the specific characteristics of the lipidic nature and are therefore important to analyse. No single analytical technique can, however, offer sufficient resolution power to separate all possible constituents. The different analytical techniques are therefore more complementary to each other than competitive. In the authors' laboratories, all these chromatographic techniques are therefore used to unravel the complexity of lipids.

The most important types of lipid analyses will be discussed below

Lipid Analysis

Separation of Fatty Acids

The type of fatty acids in fats, oil and waxes determine the physical (melting and boiling point, viscosity) and chemical (nutritional, fragrance) properties of the product. Therefore the determination of the fatty acid composition is often the first step in lipid characterization. Fatty acids need to be separated according to chain length, unsaturation and in some cases also according to the location of the unsaturation and the *cis*/*trans* configurations. For fatty acid profiling, CGC is the most widely used technique. The lipids are saponified and methylated into the fatty acid methyl esters (FAMEs) before analysis. In comparison to CGC, no other technique can provide the same resolution per unit of time. Christie has given an overview of GC separations of fatty acids,

including the determination of *trans* isomers. For the analysis of *cis*/*trans* isomers, HPLC or SFC on silver-doped columns or argentation HPLC using a mobile phase containing silver ions offers, however, more powerful alternatives.

Analysis of Triglycerides

The analysis of oils and fats without saponification is becoming more and more important as it provides typical fingerprints for each oil and fat. The triglycerides need to be separated according to the number of carbon atoms in the fatty acid chains (carbon number, (CN), separation). In a carbon number separation, the tristearin (SSS), triolein (OOO) and trilinolein (LLL) elute as one peak. CGC and cSFC can perform carbon number separations. In addition, it is also interesting to differentiate triglycerides according to unsaturation. For this type of analysis, CGC, HPLC, SFC and CEC have been used.

Profile of Mono-, Di- and Triglycerides

Additional information is also obtained by the analysis of mono- and diglycerides. This is especially important for the characterization of emulsifiers and food additives. Several emulsifiers are esters of fatty acids or fatty alcohols and glycerol, sorbitol, sorbic acid, tartaric acid or lactic acid. Also some polymer additives (slip and antistatic agents) are based on mono- and diglycerides. Often, only saturated fatty acids are used here, and a carbon number separation is sufficient. For this analysis, also CGC, HPLC and SFC can be applied.

Sterol and Sterol Ester Analysis

Sterol and sterol esters are minor constituents in oils and fats, but they are important for the quality of the products. Olive oil quality, for instance, can be monitored by the analysis of the sterol profiles. Sterols and sterol esters can be analysed by CGC, HPLC and SFC. Since the relative concentrations of sterolic compounds are low (ppm = $mg\,kg^{-1}$), prefractionation and enrichment are needed. Prefractionation can be done by classical techniques, such as saponification and liquid–liquid extraction, column chromatography or solid-phase extraction. Recently HPLC and SFC fractionation have been developed and the application of these techniques allows automation and on-line coupling to GC or GC-MS.

Phospholipids

Phospholipids form a special class of lipids. They are composed of a glycerol molecule substituted by one or two fatty acids and one additional polar group. The latter group can be choline (phosphatidylcholine or lecithin), ethanolamine, serine or inositol. Phospholipids are very polar and ionic. None the less, the fatty acid chains give to phospholipids hydrophobic properties, making them very useful as emulsifiers. The analysis of intact phospholipids by CGC or SFC is not possible due to the limited temperature stability of the solutes. Phospholipids can only be analysed by HPLC or MEKC.

Other Oleochemicals

According to the definition of Christie, other organic compounds also containing fatty acid chains can be considered as lipids. This includes waxes (mostly esters between long-chain fatty acids and long-chain fatty alcohols), ethoxylated alcohols and glycolipids. Depending on the molecular weight, CGC, HPLC or SFC can be used.

In this article examples of separation by SFC are presented for the classes of organic solutes mentioned. An overview of the nomenclature used in lipid analysis is given in the Appendix.

Supercritical Fluid Chromatography

Supercritical fluid chromatography (SFC) is a separation technique similar to gas and liquid chromatography, but using a supercritical fluid as mobile phase. If a liquid or a gas is used above its critical temperature and pressure, it changes to a supercritical fluid. The characteristics of supercritical fluids are intermediate between those of gases and liquids. A supercritical fluid can be considered as a dense gas. The lower viscosity and high diffusivity in comparison to a liquid make supercritical fluids interesting for chromatography (faster stationary phase–mobile phase mass transfer), while the higher density, in comparison to a gas, allows the solubilization and transport of the solutes through the column at lower temperatures. Moreover, the solubilization power can be modified by changing the density through temperature and/or pressure. Selective extraction (supercritical fluid extraction) and gradient supercritical fluid chromatography are possible. The low critical temperature (31.3°C) and low critical pressure (72.9 atm), together with the low toxicity and high availability, make carbon dioxide (CO_2) the only practical usable supercritical fluid.

SFC was first performed using packed columns (pSFC). Only in the late 1980s did a first generation of dedicated SFC equipment become available. At that time, however, most SFC research work was focusing on capillary SFC (cSFC) using ultranarrow bore columns (25–100 μm i.d.), pure carbon dioxide as mobile phase, (in most cases) a syringe type pump,

a fixed restrictor and typically a GC detector (mostly FID). In this article, some examples of lipid analysis by cSFC separations will be given. In the mid 1990s, a second generation of SFC instruments became available. These instruments are more dedicated to pSFC and consist of a reciprocating supercritical fluid pump, a modifier pump (modifiers are not used with capillary columns), a variable restrictor and typically an HPLC detector. pSFC has been used more successfully than cSFC for lipid analysis. Detailed information on SFC theory and instrumentation is given in Anton and Berger. See also Further Reading.

Capillary Supercritical Fluid Chromatography for the Analysis of Lipids

The analysis of neutral lipids, such as triglycerides, is one of the first successful applications of cSFC. Using an apolar capillary column (SE-54, 5% phenylmethylsilicone), good carbon number separations are obtained. This is illustrated in **Figure 1** with the analysis of palm kernel oil.

The triglycerides from CN 28 (combination of, for instance, two decanoic acid and one octanoic acid chain) to CN 54 (combination of three C_{18} fatty acids) are separated in a 30 min analysis time. For most applications, cSFC and high-temperature capillary gas chromatography (HT-CGC) provide similar quantitative data. In HT-CGC, injection and column temperature programming are however, critical, and it has been observed that highly unsaturated triglycerides (trilinolein, trilinolenin) tend to polymerize in the column at 330–360°C. cSFC offers an advantage in this respect since the analysis temperature is much lower (100–150°C). In cSFC with FID detection, the response factors of saturated and unsaturated triglycerides are very similar and calibration is easy. Especially for the analysis of oils with a high degree of unsaturation (e.g. fish oils containing mostly 22 : 6 n-3 docosahexaenoic acid and 20 : 5 n-3 eicosapentaenoic acid), cSFC offers an advantage over HT-CGC although complete separation of the lipids is not possible without a multidimensional approach.

The analysis of triglycerides according to carbon number and unsaturation, is much more difficult. The highest resolution can be obtained using HT-CGC on a diphenyldimethylsilicone phase (more than 35% phenyl substitution, e.g. OV-17, HP-50 +). As demonstrated by Geeraert, the combination of the high efficiency of the capillary column with the high selectivity of the stationary phase at 330–360°C, gives a detailed triglyceride profile. cSFC using polar cyanopropyl silicone columns (25% cyano substitution,

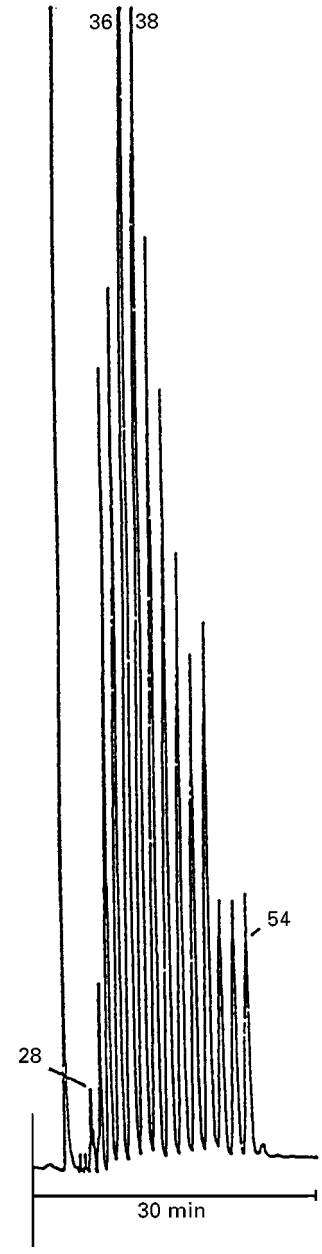


Figure 1 Carbon number separation of palm kernel oil by cSFC. Column: 10 m × 100 μm i.d × 0.2 μm SE-54. Temperature: 170°C. Pressure programmed from 19 to 29 MPa in 30 min.

e.g. OV-225, SB cyano-25) has also been used for this separation. A separation according to unsaturation of a mixture of triglyceride reference compounds is given in **Figure 2**.

For natural lipid mixtures, this separation is, however, insufficient to resolve all possible fatty acid combinations and several co-elutions occur. The lower resolution obtained with cSFC in comparison to HT-CGC is a result of the strong dependence of the column efficiency on the supercritical fluid mobile phase velocity. Although a 10 m L × 50 μm i.d.

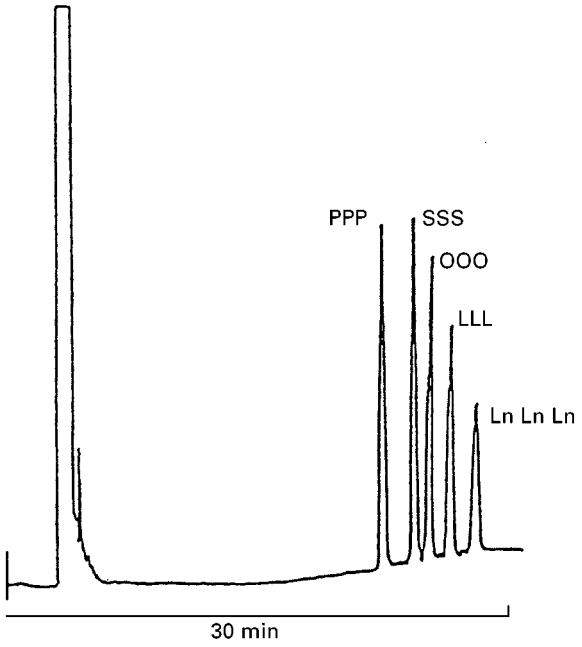


Figure 2 cSFC analysis of triglyceride standards on a polar column. Column: 10 m × 100 μm i.d. × 0.1 μm OV-225. Temperature: 150°C. Pressure programmed from 15 to 30 MPa at 0.5 MPa min^{-1}.

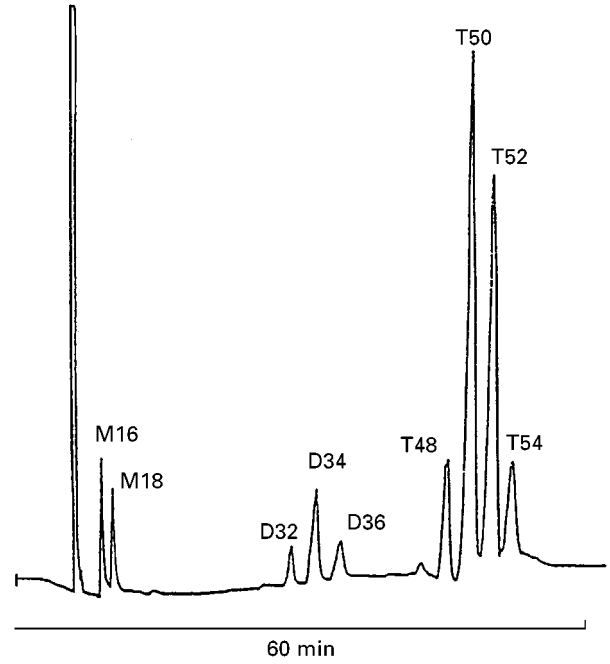


Figure 3 cSFC analysis of palm oil mono- (M), di- (D) and triglycerides (T). Column: 20 m × 100 μm i.d. × 0.2 μm SE-54. Temperature: 150°C. Pressure programmed from 15 to 25 MPa at 1 MPa min^{-1}, then to 30 MPa at 0.1 MPa min^{-1}.

column, theoretically should correspond to an effective plate number (N) 200 000, this efficiency cannot be applied in practice. The optimum linear velocity is only 0.2 cm s^{-1} and this would result in a 83 min void time and a 15 hr analysis time for a solute with capacity factor $k = 10$. In practice, the narrow bore columns are used in cSFC at much higher velocities, resulting in effective plate numbers smaller than 10 000. This is even lower than in pSFC (see below). H.J. van Oosten has published a detailed study on the qualitative and quantitative aspects of cSFC for the analysis of triglycerides.

Another interesting application of cSFC is the separation of mono-, di-, and triglycerides. For GC analysis, the mono- and diglycerides need derivatization into the trimethylsilyl derivatives. With SFC, they can be analysed without derivatization. A typical chromatogram is shown in **Figure 3**.

Glycerol monopalmitate (M16), glycerol monostearate (M18), glycerol dipalmitate (D32), palmitoylstearoylglycerol (D34), glycerol distearate (D36) and the triglycerides are well separated according to their carbon number. For complex mono-, di- and triglyceride mixtures, such as some types of emulsifiers, the higher resolving power of HT-CGC is, however, needed.

cSFC has also been used for the analysis of waxes, sterols and sterol esters and ethoxylated alcohols. An example of a beeswax analysis is given in **Figure 4**.

The analysis of phospholipids by cSFC has also been described. For this analysis, the phospholipids are derivatized using diazomethane methylation (phosphoric acid group), acylation (amine or alcohol functionality) and/or demethylation (quaternary ammonium group). Using an apolar column, the phospholipids could be separated according to the carbon number. Group-type separations of phospholipids could not be realized by cSFC.

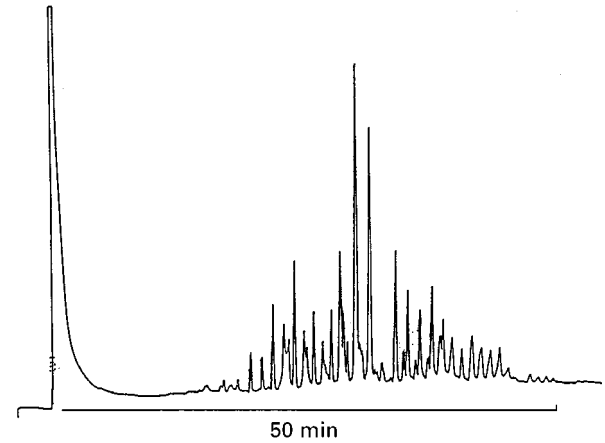


Figure 4 cSFC analysis of beeswax. Column: 10 m × 50 μm i.d. OV-1. Temperature: 100°C. Pressure programmed from 10 MPa (7.5 min) to 30 MPa at 0.4 MPa min^{-1}.

Although interesting separations could be obtained, cSFC is not widely used in routine laboratories for lipid separations. The main restriction is the robustness of the instrumentation. Column installation, restrictor maintenance and injection problems make state-of-the-art cSFC insufficiently robust for routine work.

Packed Column Supercritical Fluid Chromatography for the Analysis of Lipids

A few years after the introduction of cSFC, a second generation of SFC instrumentation was introduced on the market. This equipment was primarily dedicated to pSFC and the performance was based on specially designed reciprocating supercritical fluid pumps and new restrictor technology. The use of modifiers extended the applicability of SFC and the variable restrictors are much more robust than fixed restrictors (used in combination with capillary columns). The same performance as standard HPLC equipment in terms of repeatability, stability and robustness was realized.

Interesting pSFC separations of lipids can be made on conventional HPLC columns. Octadecyl silica (ODS), the most universal HPLC phase (reversed phase HPLC) can also be used in pSFC. The term 'reversed phase supercritical fluid chromatography (RP-SFC)', however, we consider inadequate, due to either the adsorption of carbon dioxide as a high-density layer or adsorption of the polar modifier on the stationary phase surface. Consequently, separations are actually achieved according to a normal phase mechanism. Therefore we prefer the term 'pSFC-ODS' for separations in supercritical conditions realized on ODS stationary phases rather than RP-SFC.

Triglycerides are separated on ODS columns according to the carbon number, but the presence of double bonds reduces retention. Separation of triglycerides by means of non-aqueous reversed phase high pressure liquid chromatography (NARP-HPLC) can be realized according to the equivalent chain number (ECN), whereby ECN = CN − [2 × NDB] in which CN is the carbon number and NDB the number of double bonds.

Separation of triglycerides using pSFC-ODS is achieved according to the separation number (SN), whereby the separation number equals carbon number (CN) minus the number of double bonds (NDB), thus SN = CN − NDB.

An example of such a separation is given in **Figure 5** for the analysis of peanut oil. In this separation, the peak at SN 48 corresponds to LLL and PLL, SN 49 corresponds to OLL and PLO and SN 50 to OLO + SLL + POO.

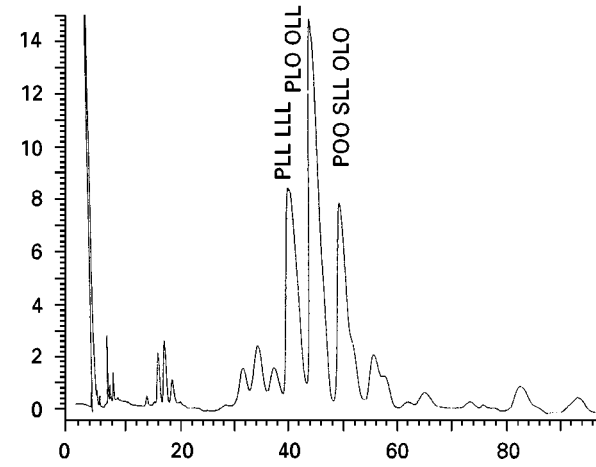


Figure 5 pSFC-ODS fractionation of triglycerides from peanut oil. Column: Adsorbosphere C18 (25 cm × 4.6 mm i.d. × 5 μm) serially coupled to Shandon ODS Ultrabase (25 cm × 4.6 mm i.d. × 5 μm). Temperature: 25°C. Flow rate: 2 mL min^{-1}. Modifier: methanol 2.5%, isocratic. Pressure: 150 bar. Detection: UV 210 nm. Injection volume: 5 μL. Sample concentration: 100 mg mL^{-1}.

In comparison to HPLC, pSFC has the advantage of a much lower pressure drop across the column due to the lower viscosity of the mobile phase. While the efficiency of the column in pSFC is equal to the efficiency in HPLC, serial coupling of several columns is feasible in pSFC. This results in much higher total effective plate number (N = sum of plate number of each column). One of the best packed column SFC separations of triglycerides has used seven 12-cm columns in series (84-cm column length in total, 5-μm ODS packing material, $N = 200\,000$). A typical separation is shown in **Figure 6**. In comparison to the separation shown in **Figure 5**, this chromatogram exhibits increased resolution. Both the efficiency and selectivity are increased in comparison to the SN separation shown in **Figure 5**. Group separation is made this time according to the ECN number, exactly as in NARP-HPLC. Resolution within a group is illustrated by the separation of PLL, POLn, OLL and OOLn, all characterized by ECN 44.

The change in the elution order can be explained either by the use of a more polar modifier (methanol–acetonitrile mixture) and by the use of subcritical elution conditions (16°C).

Another very interesting separation is obtained by pSFC using a silver-doped stationary phase. On this stationary phase, separations according to unsaturation and to some extent according to the geometrical configuration are obtained. The separation mechanism can be explained by two processes: (1) the over-

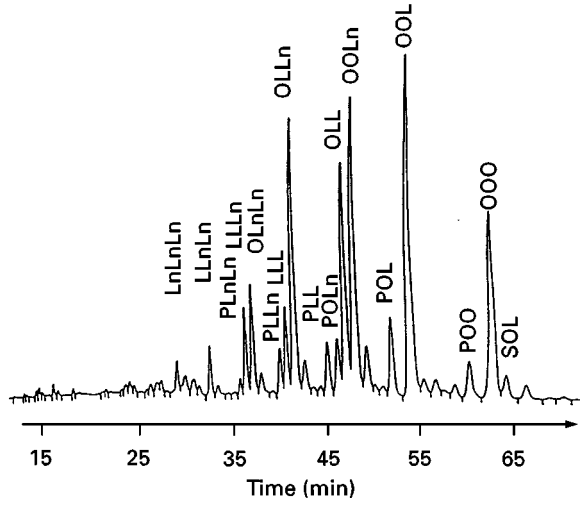


Figure 6 pSFC-ODS separation of triglycerides from rapeseed oil. Column: Hypersil ODS (12 cm × 4.6 mm i.d. × 5 μm) seven columns serially. Temperature: 16°C. Flow rate: 3 mL min^{-1}. Modifier: acetonitrile–methanol mixture 9 : 1, 6%, isocratic. Pressure: 100 bar. Detection: UV 210 nm. (Reproduced from Leselier E and Tchapla A (1996) Mise au point de l'analyse des triglycerides en chromatographie subcritique sur colonnes remplies. *Proceedings du 3ème Colloque sur les Fluides Supercritiques, Grasse, France*, pp. 115–126.)

lapping of the π orbitals belonging to the unsaturated site of the solute with the 5s orbital of the silver ion and (2) the interaction between an antibonding π* orbital of the unsaturated site of the solute with a 4d-filled orbital of the silver ion.

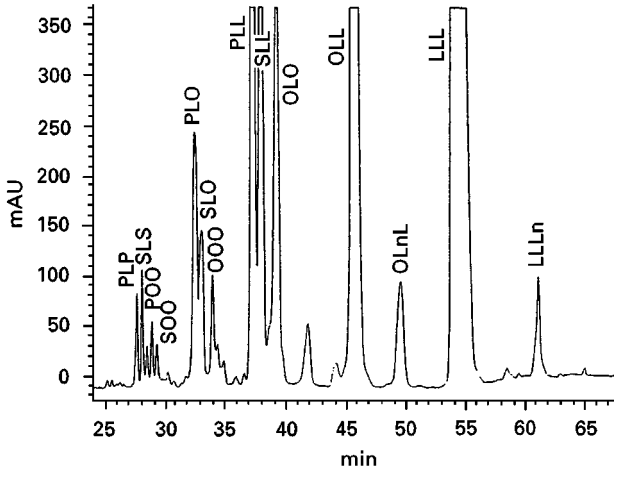


Figure 7 pSFC-SI separation of triglycerides in sunflower oil. Column: Silver-loaded Nucleosil 100-5 SA (25 cm × 4.6 mm i.d. × 5 μm). Temperature: 65°C. Flow rate: 1 mL min^{-1}. Modifier: acetonitrile–isopropanol mixture 6 : 4, programmed from 1.2% (2 min) to 7.2% (28 min) at 0.3% min^{-1}, then to 12.2% at 0.54% min^{-1}, 2.5%. Pressure: programmed from 150 bar (2 min) to 300 bar at 1.5 bar min^{-1}. Detection: UV 210 nm. Injection volume: 5 μL. Sample concentration: 100 mg mL^{-1}.

Recently, stable silver ion HPLC columns could be made by using silica-based cation exchange columns as support for silver ions. The ions are linked to the silica support via ionic bonds to phenylsulfonic acid groups chemically bonded to the silica.

Such columns have been successfully used in our laboratory both in HPLC and in pSFC. In pSFC, excellent triglyceride separations are obtained. This is illustrated in **Figure 7**, showing the analysis of sunflower oil. Within each group with the same number of double bonds, an additional separation according to chain length is observed. When solutes are characterized by the same number of double bonds, a higher retention corresponds to a higher carbon number, e.g. PLL elutes before SLL. Finally, an additional separation is obtained between lipids with the same number of double bonds and carbon number, but containing a different number of unsaturated fatty acids. Thus, SLL, containing only two unsaturated fatty acids elutes before OOL, which contains three unsaturated fatty acids.

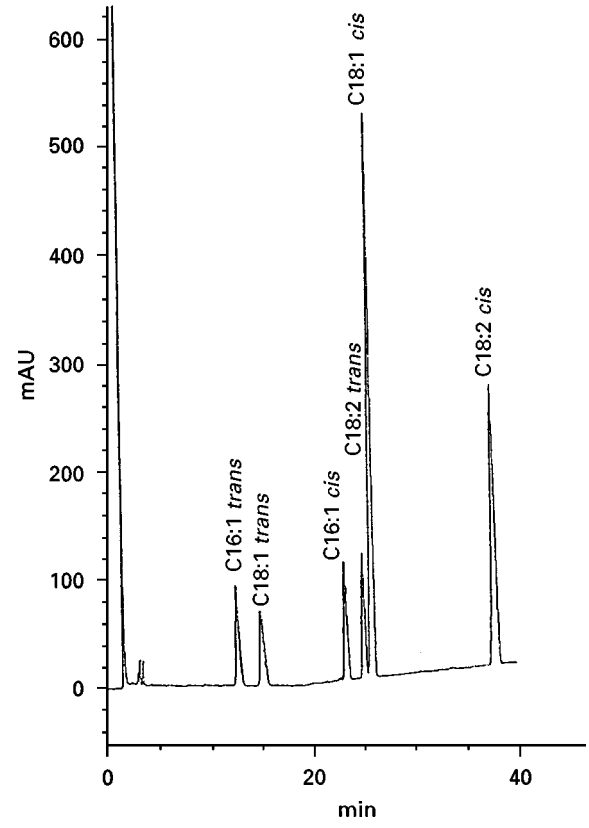


Figure 8 pSFC-SI separation of a standard mixture of geometrical isomers of fatty acids as methyl esters (FAMEs). Column: Silver-loaded Nucleosil 100-5 SA (25 cm × 4.6 mm i.d. × 5 μm). Temperature: 80°C. Flow rate : 2 mL min^{-1}. Modifier: acetonitrile–isopropanol mixture 6 : 4, programmed from 0.5% (15 min) with a gradient of 0.1% min^{-1}, 2.5%. Pressure: 250 bar. Detection: UV 210 nm. Injection volume: 5 μL.

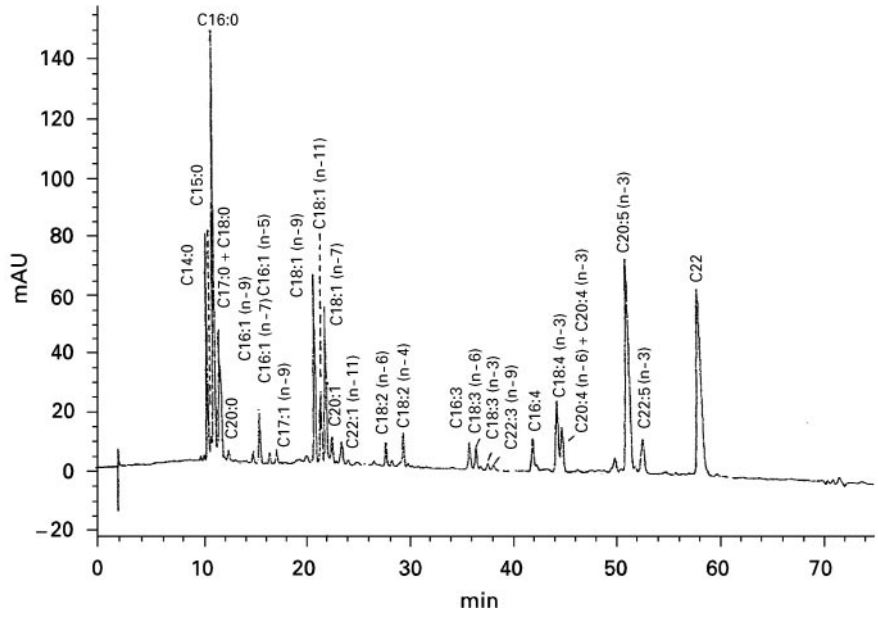


Figure 9 pSFC-SI separation of fatty acids as phenacyl esters (FAPEs) from sardine oil. Column: Silver-loaded Nucleosil 100–5 SA (25 cm × 4.6 mm i.d. × 5 μm). Temperature: 80°C. Flow rate: 2 mL min^{-1}. Modifier: acetonitrile–isopropanol mixture 6 : 4, programmed from 1% (2 min) to 41% at 0.2% min^{-1}. Pressure: 250 bar. Detection: UV 254 nm. Injection volume: 5 μL.

As can be deduced from Figures 5 and 7, the pSFC-ODS separation and the silver ion pSFC separations are complementary to each other. Very detailed triglyceride analysis can therefore be obtained by using a multidimensional approach. First a SN separation is obtained on an ODS column. The fractions of different SN number are collected and re-analysed by silver ion SFC. pSFC is especially suitable for fraction collection, since the largest part of the mobile phase is a gas after decompression and concentrated fractions are obtained.

On silver-doped stationary phases, a separation of geometrical isomers of fatty acids can also be achieved. In **Figure 8**, a standard mixture of C16:1 *cis–trans*, C18:1 *cis–trans* and C18:2 *cis–trans* fatty acid methyl esters (FAMEs) was separated. In comparison to cSFC, detection in pSFC is normally done by UV detection, because of the use of modifiers which are not compatible with FID detection. In comparison with HPLC, pSFC allows the use of low wavelengths. In the case of fatty acids, derivatization of the carboxyl group with phenacyl bromide (2-bromoacetophenone) allows detection at higher wavelengths which results in higher sensitivity. **Figure 9** shows the separation of fatty acids from sardine oil as phenacyl ester derivatives (fatty acid phenacyl esters, FAPEs) on a silver-doped stationary phase.

Another very interesting detector for pSFC is the evaporative light-scattering detector. This detector is especially useful in lipid analysis since the response factors are less dependent on the number of double bonds and very similar for saturated and unsaturated lipids.

Packed-column SFC has also been applied to other oleochemicals including sterols, sterol esters and waxes. Fractionation of free sterols from the complex matrix of vegetable oils can be achieved in a short analysis time by pSFC on an aminopropyl silica-gel (APSG) column as shown in **Figure 10**. This separation, in combination with collection of the sterol

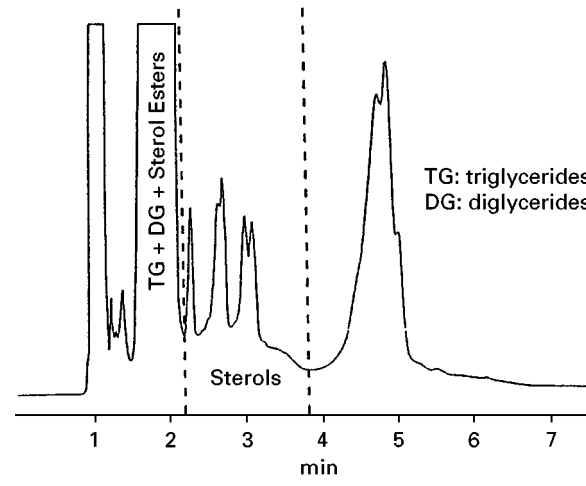


Figure 10 Isolation of the sterolic fraction in sunflower oil by pSFC. Column: Aminopropyl silica-gel APSG (20 cm × 4.6 mm i.d. × 5 μm). Temperature: 70°C. Flow rate: 2 mL min^{-1}. Modifier: methanol 10% isocratic. Pressure: 150 bar. Detection: UV 210 nm. Injection volume: 5 μL. Sample concentration: 10%.

fraction and subsequent CGC–MS analysis, was used for the characterization of vegetable oils.

Future Outlook

The next step in the use of pSFC in the analysis of oils, fats and waxes is interfacing the technique to spectroscopic detectors such as mass spectrometry or nuclear magnetic resonance spectrometry.

Supercritical fluid chromatography interfaced to mass spectrometry (SFC–MS) has already become a valuable technique in the hands of specialists. State-of-the-art pSFC–MSD interfacing has recently been reviewed by Combs *et al*.

The separation of triglycerides in vegetable oils by interfacing pSFC on a silver-doped stationary phase to atmospheric pressure chemical ionization–mass selective detection (APCI–MSD) has been described. The coupling was made using a commercially available LC interface, without any modification. No loss of resolution was noted, while sensitivity was 100 times higher compared to UV detection. The molecular $[M–H]^+$ ions could be elucidated in the mass spectra. However, the lower the degree of unsaturation, the more fragmentation occurred. Elucidation of the position of the fatty acids on the glycerol backbone (POP versus PPO for example) was feasible.

The combination of pSFC with NMR also offers a bright perspective. The mobile phase, mainly consisting of carbon dioxide, does not interfere with the ^{1}H spectra. pSFC-NMR has been reviewed by Albert.

Last, but not least, the preparative-scale features of SFC are worth mentioning. Process-scale pSFC has been applied for the production of high purity ω-3 fatty acids and their ethyl esters from fish oils. A preparative pSFC approach for the production of pure eicosapentaenoic acid has been described, highlighting again its tremendous economic impact.

Appendix: Nomenclature in Lipid Analysis

For carbon number separations, triglycerides are coded as Tnn, indicating a triglyceride with a total of nn carbon atoms in the fatty acid chains (sum of three fatty acids). T54 can thus be tristearin, triolein or another combination of three C_{18} fatty acids or C_{16}, C_{18}, C_{20}. In the same way, monoglycerides are indicated as Mnn with nn the number of carbon atoms in the fatty acid and diglycerides are indicated by Dnn, with nn the sum of the carbon atoms in the two fatty acid chains.

For detailed separations, the triglycerides are coded XYZ, indicating the fatty acid substitutes in the molecule. Abbreviations for common fatty acids are listed in **Table 1**. SLO, for instance, corresponds to a triglyceride with a stearin–linolein–olein fatty acid combination.

Table 1 Abbreviations used for common fatty acids

Abbreviation	*Fatty acid name*	*Carbon number : number of double bonds*
P	Palmitic acid	16 : 0
S	Stearic acid	18 : 0
O	Oleic acid	18 : 1
L	Linoleic acid	18 : 2
Ln	Linolenic acid	18 : 3

Table 2 Shorthand designation used for common fatty acids

Systematic name	*Trivial name*	*Shorthand designation*
cis-9-Tetradecenoic acid	Myristoleic acid	C14 : 1 (n-5)
cis-9-Hexadecenoic acid	Palmitoleic acid	C16 : 1 (n-7)
cis-6-Octadecenoic acid	Petroselinic acid	C18 : 1(n-12)
cis-9-Octadecenoic acid	Oleic acid	C18 : 1 (n-9)
cis-11-Octadecenoic acid	*cis*-Vaccenic acid	C18 : 1 (n-7)
cis-9-Eicosenoic acid	Gadoleic acid	C20 : 1 (n-11)
cis-11-Eicosenoic acid	Gondoic acid	C20 : 1 (n-9)
cis-13-Docosenoic acid	Erucic acid	C22 : 1 (n-9)
cis-15-Tetracosenoic acid	Nervonic acid	C24 : 1 (n)

For detailed separations of fatty acids, each analyte is identified as Cmm : p $(n - q)$, where mm corresponds to the number of carbon atoms in the fatty acid molecule, p is the number of double bonds and q is the number of carbon atoms from the double bond in the terminal region of the molecule. C 18:1 (n-9), for instance, is 9-octadecenoic acid (one double bond in 9 position from terminal side of molecule). The relation between the systematic name, the trivial name and the short designation mentioned above for common fatty acids in given in **Table 2**.

Further Reading

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OILS: EXTRACTION BY SOLVENT BASED METHODS

See **III / FATS / Extraction by Solvent Based Methods**

OLIGOMERS: THIN-LAYER (PLANAR) CHROMATOGRAPHY

See **III / SYNTHETIC POLYMERS / Thin-Layer (Planar) Chromatography**

ON-LINE SAMPLE PREPARATION: SUPERCRITICAL FLUID EXTRACTION

J. M. Levy, Levytech, Gibsonia, PA, USA

Introduction

Over the past few years, there have been several advances in the use of new sample-preparation strategies prior to chromatographic analyses. These include supercritical fluid extraction (SFE), solid-phase microextraction (SPME) and accelerated solvent extraction (ASE). Each of these techniques is relatively new and will be used in more analytical strategies. SPME, for example, stands out in the realm of sample preparation in that the technique is solventless. ASE is also particularly exciting since the technique represents a modern version of long-established Soxhlet extractions. Therefore, by elevating temperatures and pressures to keep the liquid solvent from vaporizing, ASE approaches can be thought of as a 'universal' sample-preparation tools. The advantage of SFE is the fact that a supercritical fluid (i.e. carbon dioxide) is utilized with its blend of liquid and gaseous properties to achieve selective extraction of target analytes without major interference (depending on the sample). Of these three techniques, only SPME and SFE can be considered selective tools and they are also the only ones that can be interfaced directly to a chromatograph. The discussion in this article will focus on the use of SFE as a viable and selective strategy for sample preparation.

SFE continues to evolve as it is applied to a more and more diverse range of sample matrices. In the early years, much emphasis was placed on using SFE for environmental methods but, this has now blossomed into the wide application of SFE for food and agricultural analyses, polymer characterization, and pharmaceutical assays.

One of the distinct advantages of SFE (besides the physical properties of liquid-like density, gas-like viscosity, no surface tension and intermediate diffusivity) is the ability to directly couple the extraction effluent from a sample matrix to an analytical chromatograph for quantitative or qualitative determination.