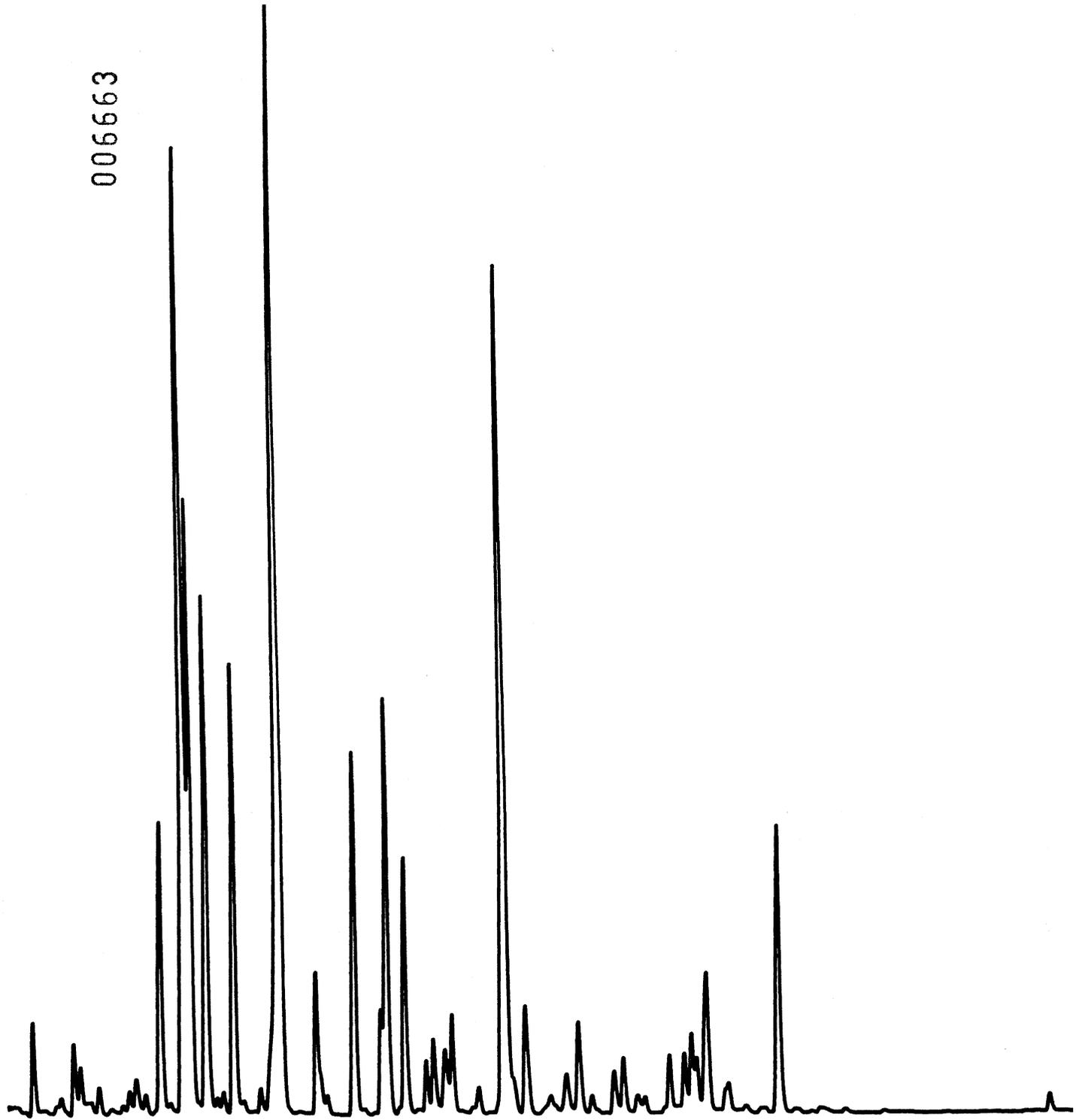


# CHROMATOGRAPHIA



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# CHROMATOGRAPHIA

# Normal-Phase High Performance Liquid Chromatographic Separation and Characterization of Short- and Long-Chain Triacylglycerols

## Key Words

Column liquid chromatography  
Evaporative light scattering detection  
APCI mass spectrometry  
Triacylglycerols

## Summary

Short- and long-chain triacylglycerols (SLCT) are a family of lipids prepared by chemical or enzymatic interesterification of triacetin, tripropionin and/or tributyrin, and long-chain ( $C_{16/18}$ ) hydrogenated vegetable oils. In this study, a normal-phase cyanopropyl high-performance liquid chromatographic (HPLC) method was developed for the separation and quantification of SLCT. The method is capable of separating SLCT mixtures, free fatty acids and the neutral lipid classes of saturated long-chain triacylglycerols, diacylglycerols and monoacylglycerols. To characterize the specific SLCT classes, a normal-phase HPLC procedure using a non-modified silica column was developed to separate the SLCT into individual isomers based on total carbon number and position of fatty acids on the glycerol backbone. Online coupling with a mass detector (LC/MS) allowed the identification of the individual triacylglycerol structures.

## Introduction

Recently, mixed short-and-long-chain triacylglycerols (SLCT) have become commercially available for use as alternative reduced-calorie fats. SLCT are prepared by sodium methoxide-catalyzed interesterification of short-chain triacylglycerols, e.g. triacetin, tripropionin and/or tributyrin, and fully hydrogenated long-chain vegetable oils using variable mole ratios of reactants. The SLCT interesterification products are a mixture of

three types of SLCT triacylglycerol structures; the first type contain two short-chain and one long-chain acyl residue (SSL), the second one short-chain and two long-chain acyl residues (LLS), and the third three long-chain acyl residues corresponding to interesterified hydrogenated vegetable oil substrate [1].

SLCT obtained by chemical interesterification have completely random TAG structures when the reaction occurs at temperatures that maintain phase homogeneity [2]. Our interest in this low-calorie class of restructured lipids is the development of directed enzymatic routes for their synthesis.

A suitable new method for the characterization of SLCT should allow detecting SLCT of randomized or non-randomized composition in the presence of other lipid classes, such as long-chain triacylglycerols (TAG), free fatty acids (FFA), monoacyl- (MAG) and diacylglycerols (DAG). Like all complex lipid mixtures, it is difficult to separate and detect most of these individual components using a single chromatographic method. The gas chromatographic (GC) determination of fatty acids as their methyl esters (FAME) is routinely used to characterize the fatty acid profile of a lipid. Since SLCT contain the short-chain acyl groups – acetyl, propionyl and/or butyryl – the quantitative recovery of FAME from the reaction medium is difficult because of their volatility and partial solubility in water [3]. In addition, the determination of individual lipid class profiles by GC analysis requires a pre-separation step with subsequent derivatization.

Lipid analyses by high-performance liquid chromatography (HPLC) has become very attractive with the introduction of the evaporative light-scattering detector (ELSD) for peak detection and quantification and by the interfacing for HPLC with a mass spectrometer for component identification. For example, Huang et al. [4] developed a method for the identification and quantification of SLCT from canola oil and triacetin/tripropionin in foods after total fat extraction by supercritical fluid extraction (SFE) with carbon dioxide. The method included online particle beam HPLC-mass

spectrometry to identify individual triacylglycerols in SFE fat samples and reversed-phase HPLC with ELSD for individual TAG quantification. This method allowed for the separation of individual SLCT and distinguished them from the medium-chain TAG of hydrogenated coconut oil in 55 minutes. The method, however, did not give data on residual TAG from the hydrogenated vegetable oil. The study also did not include SLCT containing butyroyl residues. Finally, the capabilities of this RP-HPLC method were rather limited for the concomitant analysis of residual FFA, DAG and MAG, if present.

Mixtures of lipid classes are more amenable to separation by silica-based normal-phase HPLC rather than reversed-phase HPLC. Christie and Hunter reported that they were unable to separate 1(3)-long-chain diacetyl TAG from their isomeric 2-acyl-*sn*-glycerol counterparts by silica or reversed-phase columns. However, they were able to separate 1(3) long-chain diacetyl TAG of defined stereochemistry on a diol-bonded phase [5]. The separation of free fatty acids (FFA) and neutral lipids was initially reported by El-Hamdy and Christie [6] on a normal-phase cyano column (NP<sub>CN</sub>) using a mobile phase gradient of methyl *t*-butyl ether (MTBE), hexane and acetic acid. Foglia and Jones subsequently obtained a complete baseline separation of all neutral lipid classes including free fatty acids, alkyl esters, TAG, DAG and MAG esters using a distinctly improved gradient program [7]. The lipid classes were quantitated by use of an ELSD. As the latter HPLC method was optimized for the compositional analysis of alkyl esters derived from fats and oils, it had to be further modified to meet our needs for the analyses of a series of SLCT samples obtained from the interesterification of triacetin and hydrogenated soybean oil from residual fatty acid esters, free fatty acids, triacylglycerols, 1,2- and 1,3-diglycerides, and 1 (2)-monoglycerides.

In this paper, we describe the analysis of SLCT. This technique provides a general approach for the characterization of neutral lipids in natural and modified lipid mixtures.

## Experimental

### Materials

As a reference standard for HPLC analysis, a Salatrim™ mixture from interesterification of hydrogenated soybean oil and triacetin and tripropionin (23SO) was obtained as a gift from Pfizer Central Research (Groton, CT, USA). The SLCT mixture analyzed in this study was produced by lipase-catalyzed reaction of hydrogenated soybean oil (Nabisco Foods, Indianapolis, IN, USA) and triacetin (Mangos and Foglia, unpublished results).

The following lipid standard, purity 99 %, was obtained from NuChek Prep, Inc. (Elysian, MN, USA): methyl stearate, tristearin, distearin, monostearin; each component was 25 % of the mixture. Stearic acid was obtained

from Sigma Chemical Co (St. Louis, MO, USA). All solvents used were HPLC grade: methyl *t*-butyl ether (MTBE) was obtained from J.T. Baker Inc. (Phillipsburg, NJ, USA) and hexane was obtained from Burdick and Jackson (Muskegon, MI, USA). Glacial acetic acid was analytical reagent grade obtained from Mallinkrodt (Paris, KY, USA). Solvents used to make up HPLC gradients were degassed by helium sparging prior to chromatography.

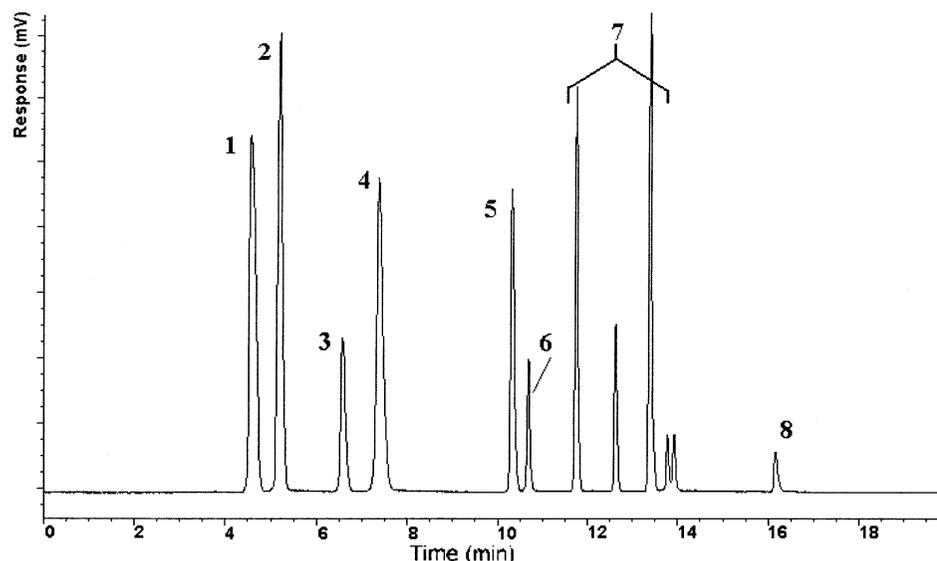
### Methods

#### HPLC Analysis of Reaction Mixtures

Over the time course of the reactions, samples containing approximately 25 mg of lipids were removed periodically and added to 1 mL of a hexane-chloroform-acetic acid mixture (190:50:1) containing tributyrin as the internal standard. The samples were then filtered through disposable Fluoropore™ PTFE membrane filters (Millipore, Bedford, MA, USA). Two drops of the filtrate were diluted in 0.5 mL hexane-MTBE-acetic acid (95:5:0.4 v:v) and analyzed by HPLC:

In method 1 (NP<sub>CN</sub>-HPLC), analyses were made on a Phenomenex (Torrance, CA, USA) cyanopropyl (CN) column (250 × 4.6 mm i.d.) with a guard column (30 × 4.6 mm i.d.) of the same phase. Separation was obtained using a binary mobile phase gradient of hexane (solvent A) and MTBE (solvent B), each containing acetic acid (0.4 %), at a flow rate of 1.0 mL min<sup>-1</sup> [7]. The gradient used in this study was modified for the purpose of separating single long-chain TAG and di- and monoacylglycerols from the newly formed short-and-long-chain TAG molecules. The initial ratio of solvent A to solvent B (100:0) was increased linearly to 90:10 over the first 6 min, then increased linearly to 20:80 over 10 min. Reequilibration of the column with solvent A was completed after 6 min. HPLC was conducted using a Hewlett-Packard (Wilmington, DE, USA) 1050 series liquid chromatograph with solvent cabinet, autosampler, and quarternary pump module. A Varex model IIA evaporative light scattering detector (Burtonsville, MD, USA) was used for analyte detection.

In method 2 (NP<sub>SiI</sub>-HPLC), separation was conducted using a Hewlett-Packard (Wilmington, DE, USA) 1050 series liquid chromatograph with solvent cabinet, autosampler, and quarternary pump module. Separations were made isocratically on a Chromegasphere Si60, 3 μm, 250 × 4.6 mm (ES Industries, Cherry Hill, NJ, USA) at a flow rate of 0.5 mL min<sup>-1</sup>. The mobile phase used was hexane : MTBE : acetic acid (82.5:17.5:0.4 %). Sample volume was 20 μL. Mass spectrometer hardware and parameters were: online LC-MS, HP Model HP5989A quadrupole mass spectrometer coupled to a HP1050 liquid chromatograph via direct liquid APCI interface HP5998A (Hewlett-Packard, Palo Alto, CA, USA) operated in the positive ion mode. Parameters: EM voltage -2906; HED voltage -10<sup>4</sup>, scan range 100-1000; threshold -50; sampling -2; Quadrupole temperature 150 °C, CapEx 100; Drying gas N<sub>2</sub>, 330 °C,



**Figure 1**  
 NP<sub>CN</sub>-HPLC chromatogram of the products from the *Carica papaya* latex-catalyzed interesterification between triacetin (TAc), adsorbed to silica powder, and hydrogenated soybean oil. **1** = stearic acid, **2** = tristearin, **3** = LLS<sub>1</sub>, **4** = LLS<sub>2</sub>, **5** = SSL<sub>1</sub>, **6** = SSL<sub>2</sub>, **7** = diacylglycerols, **8** = monoacylglycerol. (S) = acetyl residue, (L) = palmitoyl or stearoyl residues.

nebulizing gas N<sub>2</sub>, 350 °C. Computer method: APCIPOS. Full mass spectra were recorded every 0.7 s in the mass range from 100-1000 over the entire elution profile and stored on the computer hard drive. Subsequently, single ion profiles were recalled from the stored data and analyzed for both molecular and fragment ions. Values for characteristic [M-RCOO]<sup>+</sup> ions were taken from the literature [8].

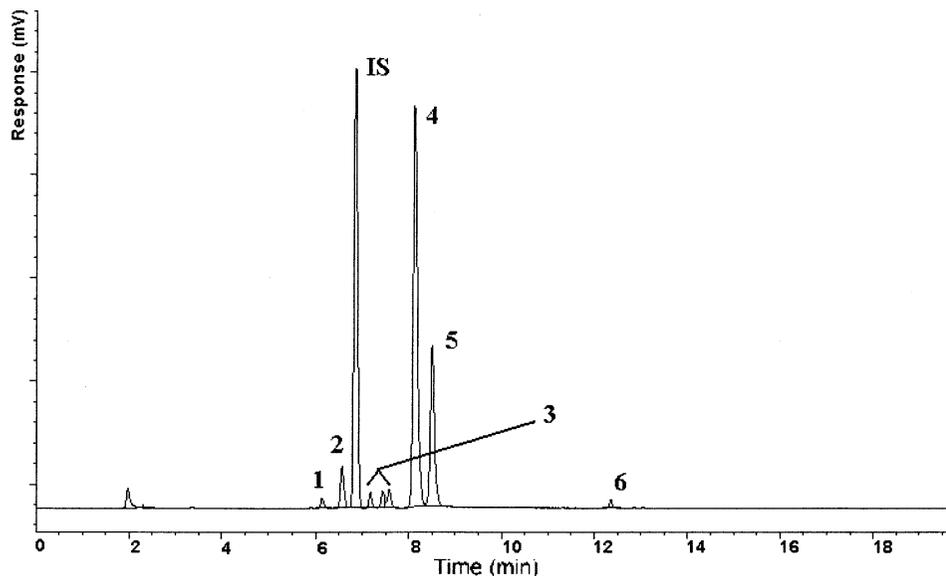
High-temperature capillary gas chromatography (HTGC) equipped with an FID detector was used for quantification of SLCT mixtures using cold on-column injection using a previously published protocol [9].

## Results and Discussion

The separation of FFA, TAG, DAG, and MAG mixtures in the *Carica papaya* latex (CPL)-catalyzed interesterification of hydrogenated soybean oil (SO) and triacetin obtained with this modified system is shown in Figure 1. Under the conditions used, there was resolution of four acetyl-containing SLCT. With the unavailability of a commercial SLCT standard the following structures were assigned to the newly formed SLCT according to their elution time and proposed composition: LLS<sub>1</sub>, LLS<sub>2</sub>, SSL<sub>1</sub>, SSL<sub>2</sub>. The two groups of SLCT of interest are represented by the abbreviations LLS<sub>i</sub> and SSL<sub>i</sub>. The first group contains one acetyl residue (S) and two stearoyl and/or palmitoyl (L) residues on the glycerol backbone, whereas the second group contains two S residues and one L residue. The SLCT are well separated from accompanying hydrolysis products of the SO substrate resulting in the appearance of long-

chain free fatty acids, diacylglycerols (DAG) and monoacylglycerols (MAG). Hydrolysis occurred because of the water present in the CPL catalyst [9]. The sensitivity of the ELSD under the optimum detector conditions, 40 °C at a nitrogen flow of 1.5 L min<sup>-1</sup>, was not sufficient enough to detect triacetin. Triacetin could only be detected at very high concentrations at a retention time (*t<sub>R</sub>*) of 12.1 min (peak not shown in Figure 1).

This NP<sub>CN</sub> HPLC system was used to optimize the enzymatic interesterification of triacetin and hydrogenated soybean oil throughout the tests to reduce hydrolytic side reactions and to increase the yield of the desired SLCT. Salatrim<sup>TM</sup> 23SO, which contains almost 90 % by weight of SSL [8], was the targeted SLCT to mimic a chemically produced SLCT composition. The latter was obtained by random interesterification of hydrogenated soybean oil and the tri-short triacylglycerols (SSS) triacetin and tripropionin in a 11:1:1 mole ratio, respectively [1]. To confirm retention times, this material was used as a reference mixture of SLCT. All TAG components in the mixture had at least one long-chain fatty acid residue. The fatty acid composition (% by weight) of this material was 26.24 % for C2:0, 3.28 % for C3:0, 7.77 % for C16:0 and 55.88 % for C18:0 by GC. The separation of the components of 23SO is shown in Figure 2. Tributyrin was used as internal standard. Compared to the reverse phase HPLC chromatographic procedure published by Huang et al. [4], where acetyl-distearoylglycerol eluted at *t<sub>R</sub>* = 50 min, the separation of the TAG molecules in 23SO with the NP<sub>CN</sub> system was achieved in 9 minutes, but in reverse elution order of SLCT.



**Figure 2**

NP<sub>CN</sub>-HPLC chromatogram of a commercial SLCT (23SO): 1 = LLS<sub>1</sub>; 2 = LLS<sub>2</sub>; 3 = mixed SSL of acetic and propionic acid; 4 = SSL<sub>1</sub>; 5 = SSL<sub>2</sub>; 6 = diacylglycerols. S = acetyl residues; L = palmitoyl or stearoyl residues; IS = internal standard (tributylin).

The chromatogram in Figure 2 shows separation of the group of mixed, propionoyl and acetyl-containing SLCT from the LLS as well as SSL triacylglycerols. The relative concentrations of the grouped SLCT structures of acetyl-containing compounds (group 1: LLL, group 2: LLS: LLS<sub>1</sub> + LLS<sub>2</sub>, and group 3: SSL: SSL<sub>1</sub>+SSL<sub>2</sub>) were found to be in good agreement with values calculated by a statistical model for random interesterification [1]. The compositional data for 23SO for SSL : LLS : LLL were found to be 88.5 : 11.1 : 0.4 % by weight, respectively.

To stretch the chromatographic system (NP<sub>CN</sub>-HPLC) to its limits and to investigate the elution behavior of SLCT, separation of a lipase-catalyzed SLCT containing acetic (A), propionic (P), and butyric acid (B) was conducted. Figure 3 shows the separation of this mixture of SLCT.

The separation of SLCT with NP<sub>CN</sub>-HPLC exhibited a distinct periodicity. The separation is apparently based on a combination of chain length of the long-chain fatty acid (LCFA) moieties (stearic and/or palmitic acid) and some type of polarity factor related to the short-chain fatty acid (SCFA) present. It was assumed that all three SCFA esterified to the glycerol backbone in the SLCT impart some diacylglycerol or monoacylglycerol character, respectively, to the TAG molecule of LLS and SSL. This observation seemed to be comparable to the frequently reported influence of acyl carbon number, reduced by the number of double bonds, on the partition number of TAG during RP-HPLC.

As expected the use of this model, developed for non-polar phases, for estimating peak retention times of SLCT that contain saturated SCFA and LCFA, did not result in a satisfactory explanation for their observed

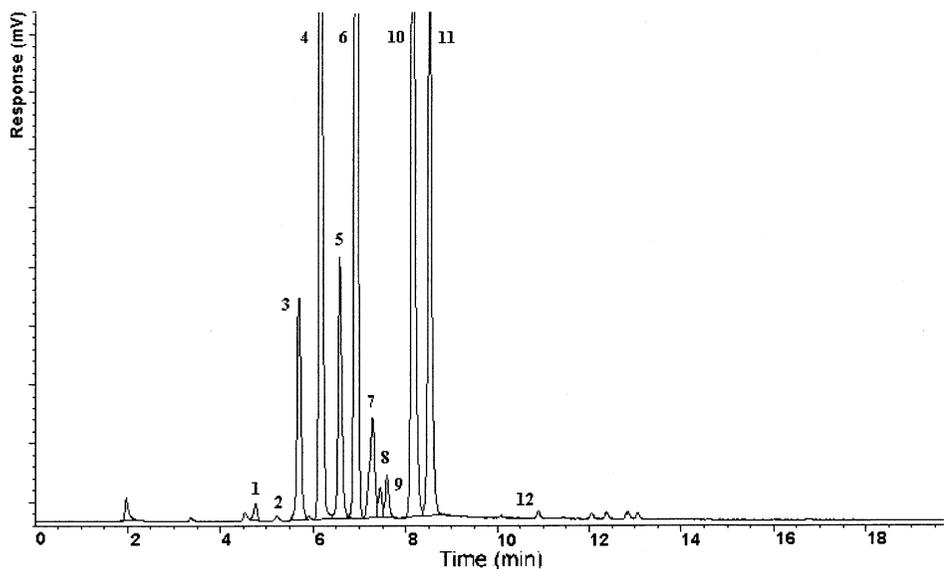
elution order. The equation especially failed in the case of the elution sequence for the following three TAG compounds: the LLS, diacetyl-stearoylglycerol, tributyrin, and the mixed SSL, acetyl-butyril-stearoylglycerol. Tributyrin eluted shortly after LLS, but surprisingly before the mixed SSL. It, therefore, can be assumed that an acetyl residue esterified to the glycerol backbone has a much larger effect on the elution characteristics of SLCT than does a propionoyl or butyryl residue.

### Quantification of Acetylated Triacylglycerols

Tributylin was used as an internal standard to correct for changes in volume during sample preparation. A SLCT calibration curve was established from the peak area ratio of total 23SO peak areas to the internal standard tributyrin (data not shown). As previously shown [4], quantification approaches utilizing HPLC with ELSD detection were not entirely linear using tributyrin but proportional to some power of the concentration of the corresponding compound due to its partial volatility under the conditions used. Absolute concentrations of SLCT, therefore, were determined using high-temperature capillary gas chromatography.

### Identification

The process of chemical interesterification results in a random distribution of the SCFA and LCFA esters onto the glycerol backbone. The molar ratios of the LLL and SSS reactants determine the relative concentration of the various possible SLCT products and their isomeric structures. The distribution of SLCT species for a ran-



**Figure 3**

NP<sub>CN</sub>-HPLC chromatogram of an SLCT obtained by enzymatic interesterification of 23SO and tributyrin. **1** = stearic acid; **2** = tristearin; **3** = LLS<sub>B</sub>; **4** = S<sub>B</sub>S<sub>B</sub>L; **5** = LLS<sub>A</sub>; **6** = S<sub>B</sub>S<sub>B</sub>S<sub>B</sub>; **7** = LS<sub>B</sub>S<sub>A</sub>; **8** = LS<sub>P</sub>S<sub>A</sub>; **9** = LS<sub>A</sub>S<sub>P</sub>; **10** = LS<sub>A</sub>S<sub>A</sub>; **11** = S<sub>A</sub>LS<sub>A</sub>; **12** = 1,3-monostearin. S<sub>A</sub> = acetyl; S<sub>P</sub> = propionyl; and S<sub>B</sub> = butyryl residues, respectively, and L = palmitoyl or stearoyl residue.

dom interesterification reaction can be precisely predicted from the mole ratio of the fatty acids present in the reaction mixture. To use the mathematical model it is necessary that excess tri-short-chain TAG (SSS) be removed from the final SLCT product. Based on the model for SLCT interesterification [1], 20 isomeric SLCT structures for 23SO were predicted to occur at 0.1 % by weight or greater. This model also predicted 9 SLCT structures having a concentration of 1 % by weight or higher. Separation on NP<sub>CN</sub> resulted in the detection of only 7 compounds. Group 3 (SSL) was expected to contain 4 isomers amounting to 73.76 % by weight of total SLCT: 1,2-diacetyl-3-stearoylglycerol (SSL), 1,3-diacetyl-2-stearoylglycerol (SLS), 1,2-diacetyl-3-palmitoylglycerol (SSL), 1,3-diacetyl-2-palmitoylglycerol (SLS), accounting for 43.25, 21.63, 5.92, and 2.96 % by weight, respectively. Due to the variability in fatty acid content in SO and small deviations of predicted vs. actual SLCT weight percentages, the exact identity of the two SSL components separated by NP<sub>CN</sub> was not possible.

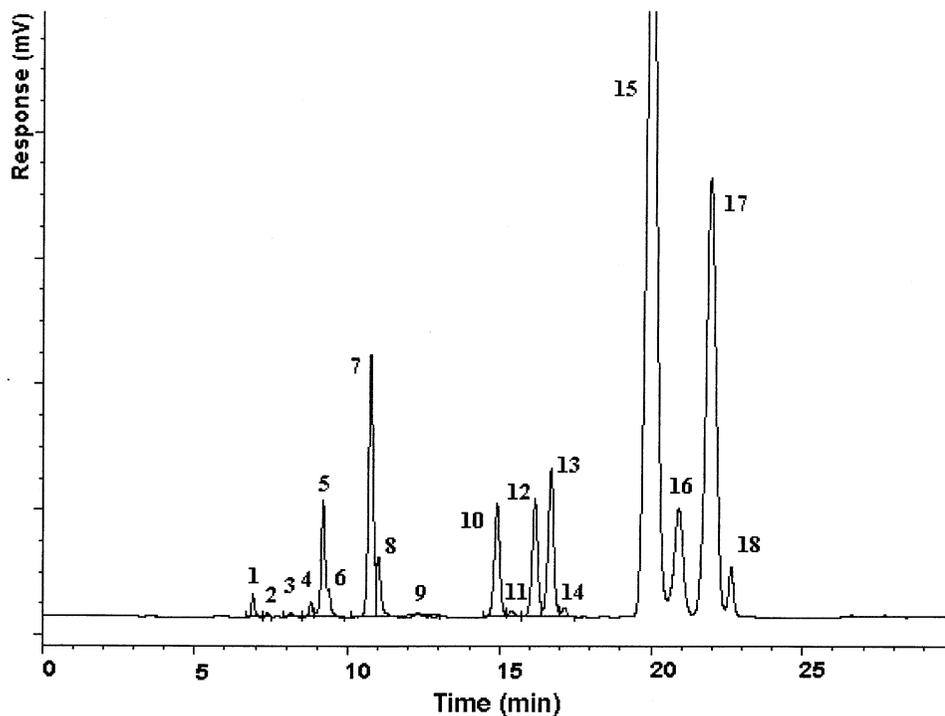
#### Separation of SLCT Isomers

Separation of the single SLCT isomer structures was achieved on a normal-phase silica column (NP<sub>Sil</sub>) using an isocratic mobile phase of hexane:MTBE:acetic acid (Figure 4). Identification was made on an online LC/MS quadrupole mass spectrometer coupled online via direct liquid APCI interface and operated in the positive ion mode. Full mass spectra were taken in the mass range from 100–1000 (*m/z*) over the entire elution profile. Single ion profiles were analyzed for both molecular and

fragment ions. Values for characteristic [M-RCOO]<sup>+</sup> ions were taken from the literature [8]. The major ions for the peaks separated by HPLC were as follows. Masses for ions [M-RCOO]<sup>+</sup> were calculated for the following diglyceride fragments: *m/z* 607, DG 36:0 (18:0–18:0); *m/z* 579, DG 34:0 (18:0–16:0); *m/z* 383, DG 20:0 (18:0–2:0); *m/z* 355, DG 18:0 (16:0–2:0); *m/z* 100, DG 4:0 (2:0–2:0).

#### Conclusion

The present high-performance liquid chromatography system using a normal-phase cyanopropyl column (NP<sub>CN</sub> HPLC) initially was developed to separate neutral lipid classes. With further modifications, we have now found that the method is additionally able to separate SLCT containing the short-chain acids butyric, propionic and acetic. The SLCT eluted in a time window between the long-chain TAG from hydrogenated vegetable oil and their respective diacyl- and monoacylglycerol derivatives. Complete separation could be achieved of the major components of SLCT mixtures that contained one or two moles of the short-chain fatty acids studied. The separation of symmetrical and asymmetrical SLCT-containing acetyl and propionoyl residues also was feasible. This is in contrast to results obtained on reversed-phase columns [4], where separation of SLCT is based on total number of carbon atoms and double bonds only. Separation of SLCT by NP<sub>Sil</sub>-HPLC combined both the power of RP<sub>ODS</sub>-HPLC and NP<sub>CN</sub>-HPLC in regard to acyl positional differences and total



**Figure 4**  
 NP<sub>SiI</sub>-HPLC chromatogram of an SLCT obtained by chemical interesterification of hydrogenated soybean oil and triacetin and tripropionin at a 1:11:1 ratio (% by weight). For peak identification, see Table I.

**Table I.** Triacylglycerol (TAG) structures for SLCT separated by NP<sub>SiI</sub>-HPLC.

Peak*	R <sub>t</sub> (min)	major ions ( <i>m/z</i> )	proposed structure
1	6.851	579, 607	18:0-18:0-16:0
3	8.124	397, 369	18:0-3:0-16:0
4	8.770	397, 607	18:0-18:0-3:0
5	9.160	383, 607	18:0-2:0-18:0
7	10.721	383, 607	18:0-18:0-2:0
8	10.980	579, 355	18:0-16:0-2:0
10	14.889	397, 173	18:0-3:0-2:0
11	15.392	369, 173	16:0-3:0-2:0
12	16.151	383, 173	18:0-2:0-3:0
13	16.680	383, 397	2:0-18:0-3:0
14	17.136	355, 369	2:0-16:0-3:0
15	20.001	383, 159	18:0-2:0-2:0
16	20.879	355, 159	16:0-2:0-2:0
17	21.917	388	2:0-18:0-2:0
18	22.609	355	2:0-16:0-2:0

\* Peak numbers correspond to SLCT found in Figure 4

acyl carbon number of SLCT. Thus, identification of SLCT is possible using NP<sub>SiI</sub>-HPLC and an online-coupled mass spectrometer.

Separation of SLCT by NP<sub>CN</sub> also is suitable for detecting low levels of unreacted triacylglycerols, free fatty acids, and di- and monoacylglycerols in edible fats and oils, as well as the broad range of lipid classes of short-

and-long-chain TAG that may be encountered in the analysis of this class of lipids. The data show that this versatile HPLC method was effective in detecting the progress of unreacted triacylglycerols and the ratio of hydrolysis in the enzyme-catalyzed interesterified mixtures.

## References

- [1] B. J. Softly, A. S. Huang, J. W. Finley, M. Petersheim, R. G. Yarger, M. M. Chrysam, R. L. Wiczorek, M. S. Otterburn, A. Manz, G. J. Templeman, *J. Agric. Food. Chem.* **42**, 461 (1994).
- [2] L. P. Klemann, K. Aji, M. M. Chrysam, R. P. D'Amelia, J. M. Henderson, A. S. Huang, M. S. Otterburn, R. G. Yarger, *J. Agric. Food Chem.* **42**, 442 (1994)
- [3] J. L. Iverson, A. J. Sheppard, *J. Am. Oil Chem. Soc.* **60**, 284 (1977).
- [4] A. S. Huang, L. R. Robinson, L. G. Gursky, R. Profita, C. G. Sabidong, *J. Agric. Food Chem.* **42**, 468 (1994).
- [5] W. W. Christie, M. L. Hunter, *Biochem. J.* **235**, 833 (1986).
- [6] A. H. El-Hamdy, W. W. Christie, *J. High Resol. Chrom.* **16**, 55 (1993)
- [7] T. A. Foglia, K. C. Jones, *J. Liq. Chrom. & Rel. Technol.* **20**, 1829 (1997).
- [8] L. Marai, A. Kuksis, J. J. Myher, *J. Chromatogr. A* **672**, 87 (1994).
- [9] T. A. Foglia, P. Villeneuve, *JAOCS* **74**, 1447 (1997).

# CHROMATOGRAPHIA

## Some important papers published during 1998

### Biomedical

Urinary Steroid Analysis of Women with Effluvia

*E. Telegdy / S. Juricskay*

A Methodological and Metabolite Identification Study of the Metabolism of

S-Carboxymethyl-L-Cysteine in Man

*G. B. Steventon*

### Development

Electrically Driven Capillary Size Exclusion Chromatography

*E. Venema / J. C. Kraak / H. Poppe / R. Tijssen*

Porous Copolymer of the Methacrylic Ester of Dihydroxydiphenylmethane Diglycidyl Ether and Divinylbenzene as an HPLC Packing

*B. Gawdzik / T. Matynia / J. Osypiuk*

Preparation and Characterization of *p*-tert-Butyl-Calix[6]arene-Bonded Silica Gel Stationary Phase for High-Performance Liquid Chromatography

*W. Xu / J.-S. Li / Y.-Q. Feng / S.-L. Da / Y.-Y. Chen / X.-Z. Xiao*

### Environmental

Two-Dimensional High Performance Liquid Chromatography for the Separation of Complex Mixtures of Explosives and their By-Products

*A. P. Köhne / U. Dornberger / T. Welsch*

Comparative Study on Determination of Fumagillin in Fish by Normal and Reversed Phase Chromatography

*J. Fekete / Zs. Romvári / I. Gebefügi / A. Ketrup*

Separation of Isomeric Polycyclic Aromatic Hydrocarbons by GC-MS: Differentiation Between Isomers by Positive Chemical Ionization with Ammonia and Dimethyl Ether as Reagent Gases

*K. Riahi / N. Sellier*

Sulfuric Acid Pretreatment for the Simultaneous GC Screening of Organochloride and Organophosphorus Pesticides in Herbal Essential Oils

*H.-R. Yoon / E.-J. Lee / M.-K. Park / J.-H. Park*

Large-Volume PTV Injection: Comparison of Direct Water Injection and In-Vial Extraction for GC Analysis of Triazines

*J. Teske / J. Efer / W. Engewald*

Fast Determination of VOCs in Workplace Air by Solid-Phase Extraction and Gas Chromatography - Mass Spectrometry

*F. Mangani / L. Lattanzi / M. Maione*

An Improved Analytical Method for Determination of Heterocyclic Amines in Chicken Legs

*B. H. Chen / D. J. Yang*

Potential of Short-Column Liquid Chromatography with Tandem Mass Spectrometric Detection for the Rapid Study of Pesticide Degradation

*A. C. Hogenboom / R. J. C. A. Steen / W. M. A. Niessen / U. A. Th. Brinkman*

Extraction of Polypropylene Additives and Their Analysis by HPLC

*H. El Mansouri / N. Yagoubi / D. Ferrier*

### Food

Determination of Heterocyclic Aromatic Amines in Fried Beefsteak, Meat Extract, and Fish by Capillary Zone Electrophoresis

*C. Mardones / L. Arce / A. Rios / M. Valcárcel*

Determination of Heterocyclic Aromatic Amines (HAA) in Commercially Available Meat Products and Fish by High Performance Liquid Chromatography - Electropray Tandem Mass Spectrometry (HPLC-ESI-MS-MS)

*E. Richling / D. Häring / M. Herderich / P. Schreier*

### Fundamental

The Effect of Conductivity Tuning in Chiral Separations by CE; Using Hydroxypropyl- $\beta$ -Cyclodextrin in Combination with Tetraalkylammonium Ions

*O. Stålborg / M. Hedeland / C. Pettersson / D. Westerlund*

Chiral Discrimination of *N*-(Dansyl)-DL-Amino Acids on Human Serum Albumin Stationary Phase: Effect of a Mobile Phase Modifier

*E. Peyrin / Y. C. Guillaume*

Comparison of Packed Capillary Solvating Gas Chromatography with Supercritical Fluid Chromatography Using Carbon Dioxide as Mobile Phase

*N. Wu / Y. Shen / M. L. Lee*

Revisiting the Problem of Correcting Retention Parameters for Pressure and Temperature in Gas Chromatography

*V. A. Davankov*

Synthesis and Characterisation of Long Chain Alkyl Stationary Phases on a Silica Hydride Surface

*J. J. Pesek / M. T. Matyska / S. Takhar*

### Pharmaceutical

High Performance Liquid Chromatography Method for the Determination of Doxycycline in Human Plasma

*D. Farin / G. Piva / I. Gozlan / R. Kitzes*

Enantiomeric Separation of Four Methylendioxyethylated Amphetamines on  $\beta$ -Cyclodextrin Chiral Stationary Phases

*F. Sadeghipour / J.-L. Veuthey*

An Improved High-Performance Liquid Chromatography Method for the Determination of Homocysteine in Human Plasma

*M. Tröbs / T. Renner / G. Scherer*

Interlaboratory Study of Analysis of Phenoxymethylpenicillin by Liquid Chromatography

*Zhu Yongxin / E. Roets / B. Trippe / Ch.-P. Christiansen / M. P. Arevalo / E. Porqueras / B. Maichel / P. Inama / S. Söderholm / J. H. McB Miller / J. M. Spieser / J. Hoogmartens*

Direct Solid-Phase Microextraction Combined with Gas and Liquid Chromatography for the Determination of Lidocaine in Human Urine

*E. H. M. Koster / N. S. K. Hofman / G. J. de Jong*

The Application of High Performance Liquid Chromatography, Coupled to Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry (HPLC-NMR-MS), to the Characterisation of Ibuprofen Metabolites from Human Urine

*E. Clayton / S. Taylor / B. Wright / I. D. Wilson*

Highly Selective Antibody-Mediated Extraction of Isoproturon from Complex Matrices

*S. J. Shahtaheri / P. Kwasowski / D. Stevenson*

Analysis of Chlortetracycline and Related Substances by Capillary Zone Electrophoresis: Development and Validation

*Y. M. Li / H. Moons / A. Van Schepdael / E. Roets / J. Hoogmartens*

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### Phases

Studies on Shape Selectivity of RP C18-Columns

*H. Engelhardt / M. Nikolov / M. Arangio / M. Scherer*

Determination of Weak (2.0-2.5) Dissociation Constants of Non-UV Absorbing Solutes by Capillary Electrophoresis

*J.-P. Mercier / Ph. Morin / M. Dreux / A. Tambute*

Structure-Driven Retention Model for Optimization of Ternary Solvent Systems in Reversed-Phase Liquid Chromatography

*W. Kiridena / C. F. Poole*

Applications of Molecularly Imprinted Materials as Selective Adsorbents: Emphasis on Enzymatic Equilibrium Shifting and Library Screening

*O. Ramström / L. Ye / M. Krook / K. Mosbach*

Ligand-Exchange Chromatographic Separation of DL-Amino Acids on Aminopropylsilica-bonded Chiral *s*-Triazines

*M. Wachsmann / H. Brückner*

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