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Article

# A Quick, Optimized Method for Routine Analysis of Essential and *Trans*-Octadecenoic Acids in Edible Fats and Oils by GLC

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**A gas chromatography procedure using a 30-m capillary column (VF-23ms) has been optimized for quick analysis (run time less than 20 min). The method is suitable for routine analysis of the fatty acid composition of edible fat and oil samples (milk, fish, vegetal and synthetic origin). The results were comparable with those obtained with a 100-m-long CP-Sil 88 column (run times between 60 and more than 100 min) in the analysis of geometric and positional isomers of polyunsaturated fatty acids. The achieved resolution of compounds from 4 to 26 carbon atoms with 0–6 double bonds, as omega-3 and omega-6 fatty acids, major *trans*-octadecenoic isomers and conjugated linoleic acid isomers, was higher than those reported in other proposed and reference methods for similar samples using short-length columns. The response factors obtained from the fatty acid composition of reference milk fat exhibited high feasibility and the inter-assay (VF23ms versus CP-Sil 88) and intra-assays based on relative standard deviation showed good accuracy, because they were lower than 10%.**

## Introduction

In Western countries, current nutritional patterns are characterized by a substantial intake of animal fat, seed oil and precooked food, resulting in diets with elevated contents in saturated and *trans* fatty acids (TFA). Several studies have shown their role in the development of certain diseases such as cardiovascular disorders, metabolic syndromes (1, 2) and some kinds of cancer (3). Nevertheless, certain fatty acids (FA) and lipid compounds do not exert a harmful effect and can improve or maintain a consumer's health (4, 5). Examples are the reported anticarcinogenic activities for butyric acid (6), conjugated linoleic acid (CLA) (7) and α-eleostearic acid (a conjugated linolenic acid isomer, CLnA) (8); antiatherogenic effects of oleic acid (9), and the relation between the omega-3 and omega-6 family (10); additionally, these compounds play an important role in the development of the infant nervous system (11) and have positive effects in certain mental diseases (12).

Ruminant *trans* fat is a naturally-occurring FA found in meat and dairy foods, in which the vaccenic acid (TVA; C18:1 11t) is the predominant *trans* monoene isomer, reaching the 50–80% of total *trans* fat. These FA have very different health effects than industrial *trans* fats, such as partially hydrogenated vegetable oils, which have been strongly associated with cholesterol and coronary heart disease (13). Over 80% of CLA (C18:2 9c, 11t; rumenic acid, RA) in ruminant fat is endogenously synthesized by $\Delta^9$-desaturase using TVA as substrate. Humans also possess the ability to desaturate TVA to RA. Nevertheless, a substantial portion of natural *trans* fatty acid content is included in the total *trans* fats calculation on nutrition labels, which is misleading for the consumer. Therefore, the availability of a rapid chromatographic method that allows the separation of different *trans* monoene isomers for further identification and quantification is crucial.

Gas liquid chromatography (GLC) is the primary technique used in the qualitative and quantitative analysis of the FA composition of edible fats and oils. The American Oil Chemists' Society (AOCS) has released several official methods (14) and recommended the CP-Sil 88 100-m-long column for the determination of FA composition in complex food lipid samples to obtain a good resolution between *cis* and *trans* isomers (15, 16). However, to accomplish this goal, long run times would needed, which is not adequate for routine analyses. In recent years, there has been renewed interest in the development and implementation of faster GLC methods (17). Currently, new capillary columns are available with lower particle size diameters, allowing considerable reduction in analysis time. In general, the proposed rapid GLC applications do not allow determination of a detailed FA profile, especially the minor and branched FA and mono-unsaturated *cis* and *trans* isomers (18, 19).

The present research work used a VF23ms 30-m-long column, and the results were compared with those attained from a 100-m CP-Sil88 column. Two temperature programs were assayed: P0, for the analysis of milk fat focused on the separation among short-chain FAs (SCFA), *trans*-octadecenoic isomers and CLA; and P1, for the analysis of vegetable and fish oils focused on the separation of long and very long-chain FAs (LCPUFA, VLCPUFA). For the optimization of this procedure, several reference fats and oils with known composition were used, as well as other consumer oils. The aim of the present work was to perform a comparative analysis using different commercially available edible fats and oils to obtain a routine chromatographic methodology with a short run time and the maximal resolution capability for *trans*-octadecenoic FA, essential FA (linoleic and linolenic acids) and CLA isomers.

## Experimental

### *Chemicals and standards*

All reagents used in the current research work were analytical grade: hexane and methanol (LabScan, Dublin, Ireland), potassium hydroxide and sodium sulphate-1 hydrate (Panreac, Barcelona, Spain) reference milk butterfat CRM-164 (Fedelco Inc., Madrid, Spain) and glyceryl tritridecanoate (internal standard for milk sample), Supelco 37 fatty acid methyl ester (FAME)

mix and triheptadecanoate (internal standard for oil samples) (Sigma, St. Louis, MO).

### *Samples*

Sunflower, safflower and soya oils were purchased from a local market; tung oil was from Sigma Aldrich, fish oil was from DSM (Kaiseraugst, Switzerland) and tonalin was provided by Cognis (Illertissen, Germany).

### *Preparation of FAMEs*

(FAMEs were prepared by base-catalyzed transmethylation of fat and oils using 2N KOH in methanol, as described by International Standard method ISO-IDF (20).

### *Gas chromatography–flame ionization detection analysis*

#### *Conventional analysis*

FAMEs were analyzed on an Agilent gas chromatograph (model 6890N, Palo Alto, CA) fitted with a flame ionization detector (FID) coupled with a CP-Sil 88 fused-silica capillary column (100 m × 0.25 mm i.d. × 0.2 μm film thickness; Chrompack, Middelburg, The Netherlands). The column temperature was held at 100°C for 1 min after injection, then 7°C/min to 170°C, held for 55 min, 10°C/min to 230°C and held 33 min. Helium was the carrier gas with an inlet pressure set at 214 kPa (30 Psig) and a split ratio of 1:20. The injector temperature was set at 250°C and the detector temperature was set at 270°C. Injection volume was 0.5 μL. Reference butterfat CRM-164 was injected to obtain response factors (RF). For quantitative purposes, tritridecanoin was spiked in reference butterfat, while in oil samples triheptadecanoate was used. Total run time was 105 min.

#### *Quick analysis*

Quick GC analyses were conducted on a Clarus 500 chromatograph (Perkin Elmer, Beaconsfield, UK) fitted with a VF-23ms, fused-silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Varian, Middelburg, The Netherlands) and FID. Two temperature programs were adapted for milk fat (P0) or for vegetable and fish oil (P1) due to the presence or absence, respectively, of SCFAs.

#### *Temperature program 0 (P0)*

The starting temperature was 80°C, 15°C/min to 130°C, then 3°C/min to 170°C and 20°C/min to 230°C, and held 5 min. Helium was the carrier gas, with a column inlet pressure set at 120.5 kPa (15 psig) and a split ratio of 1:50. The injection volume was 0.5 μL. The total run time was 26.7 min, but less than 20 min for the last eluting compound.

#### *Temperature program 1 (P1)*

The column temperature was held at 160°C for 14 min, then 45°C/min to 210°C and held 5 min. Helium was the carrier gas, with a column inlet pressure set at 120.5 kPa (15 psig) and a split ratio of 1:50. The injection volume was 0.5 μL. The total run time was 20.3 min.

### *Validation parameters*

Intra-assay and inter-assay precision (VF23ms versus CP-Sil 88) were assessed by the relative standard deviation (RSD) relative to three replicates per sample, performed by the same operator, and results were accepted if they were under 10% (21).

## Results and Discussion

### *Comparison of the qualitative results*

Figure 1A shows the PUFA region of CRM164 reference milk fat, using the CP-Sil 88 column. In this column, the C18:1 *trans* major isomers eluted as C18:1 6t-8t, C18:1 9t, C18:1 10t, C18:1 11t (TVA) and C18:1 12t-14t. It was also possible to identify the minor *cis*-octadecenoic isomers C18:1 11c, C18:1 12c, C18:1 13c, C18:1 14c (the latter coeluting with C18:1 16t) and C18:1 15c. The essential linoleic acid (LA; omega6) was detected, as was its minor isomers: C18:2 t, t non-methylene interrupted double bonds (NMID), C18:2 9t, 12t, C18:2 9c, 12t, C18:2 11t, 15c, C18:2 9t, 12c and C18:2 9t, 15c. For the CLA region, in addition to the presence of RA, it was possible to identify and quantify different CLA isomers such as C18:2 11t, 13c, C18:2 13t, 15t, C18:2 12t, 14t, C18:2 11t, 13t, C18:2 9t, 11t and C18:2 *trans*, *trans*. The omega-3 fatty acid, linolenic acid (LnA) eluted just before CLA.

With the VF23ms column (Figure 1B) and using the temperature program P0, *trans*-octadecenoic isomers partially overlapped in three peaks were identified as C18:1 4t-9t, C18:1 10t and TVA, while the minor compounds of C18:1 *cis* were resolved as C18:1 11c, C18:1 12c and C18:1 13c-14c. The isomers of CLA were differentiated by the elution order of the conformations *trans*, *trans*; *cis*, *trans*; *trans*, *cis*.

Figures 2 to 7 show the chromatogram profiles from the FA analysis of vegetable and fish oils with the CP-Sil 88 column in comparison with the VF23 column using the P1 temperature program. Total run time was less than 17 min. C18:1 *trans*-octadecenoic fatty acids were detected in none of the vegetable oils, while C18:1 5t-9t and C18:1 10t were found in fish oil. With the VF23ms column, minor LA isomers were identified (Figures 2B–7B) C18:2 t, t NMID and C18:2 9t, 12t in tonalin oil, C18:2 9c, 13t in all the assayed samples, C18:2 9c, 12t (not detected in tung and tonalin oils) and C18:2 11t, 15c (only in tonalin oil). The CLA isomers eluted in four peaks (RA, C18:2 10t, 12c, total C18:2 c, c and C18:2 t, t). In addition, to arachidonic (AA), eicosapentanoic (EPA), docosapentanoic (DPA) and docosahexanoic (DHA) acids, two CLnA isomers (C18:3 9c, 11t, 13t and C18:3 9t, 11t, 13t) were determined. These compounds were identified according to standard FAME mix Supelco 37 and previous studies reporting the composition of rich CLA and tung oils (22, 23).

Elsewhere, other authors have pointed out the problems with diminishing the chromatographic analysis time without compromising the resolution of FA. Destaillats and Cruz-Hernandez (18) carried out the analysis of milk fat within 4 min using columns of 10 m, with hydrogen as carrier gas. In that study, the C18:1 *trans* and *cis* isomers were not separated and co-eluted in one single peak, while from the CLA isomers, only RA was detected. Other research works using 40-m capillary columns reported chromatographic run times of 26–30 min for the analysis of human milk fat and plasma, not detailing the

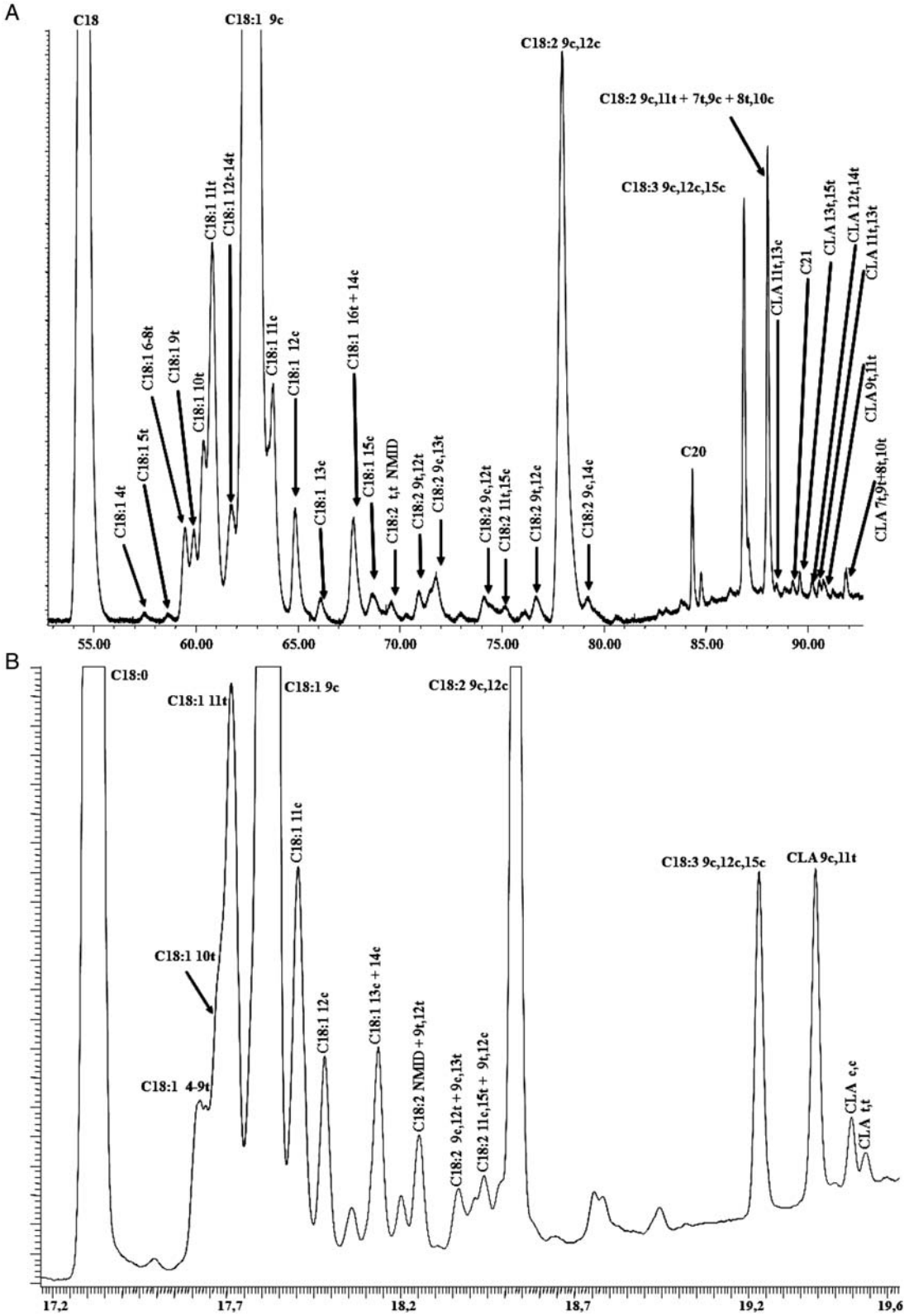


**Figure 1.** Fatty acid profile of the C18:1 and C18:2 region of a milk fat sample: 100-m CP-Sil 88 column (A); 30-m VF23ms column (B).

composition of C18:1 *trans* and *cis* isomers, while CLA isomers were identified and quantified as RA, C18:2 11c, 13t, C18:2 10t, 12c and C18:2 t, t CLA (24, 25). A recent study comparing CP-Sil 88 with Omegawax-250 columns (50 m, > 50 min run time) showed the analysis of FA standard mixtures in which the *cis* and *trans*-octadecenoic overlapped, and although a separation

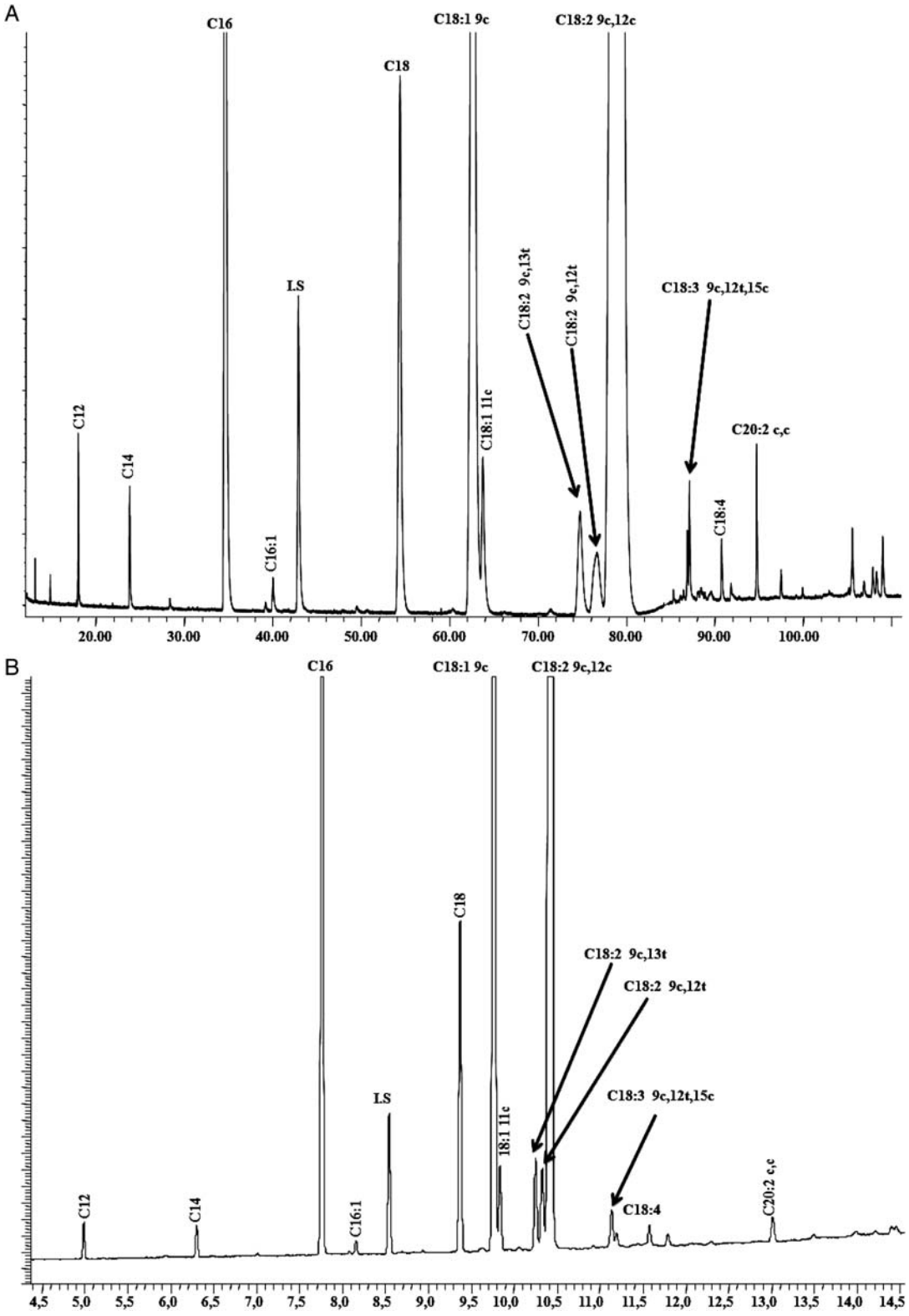


**Figure 2.** Fatty acid profile of safflower oil sample: 100-m CP-Sil 88 column (A); 30-m VF23ms column (B).

between C18:2 *trans*, *trans* and *cis*, *cis* isomers was achieved, the *cis*, *trans* and *trans*, *cis* coeluted, which was a situation similar to that for CLA (26).

The AOAC official method Ce 1h-05 for the analysis of saturated (SFA), *cis*-, *trans*-, monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in oil and non-ruminant fats

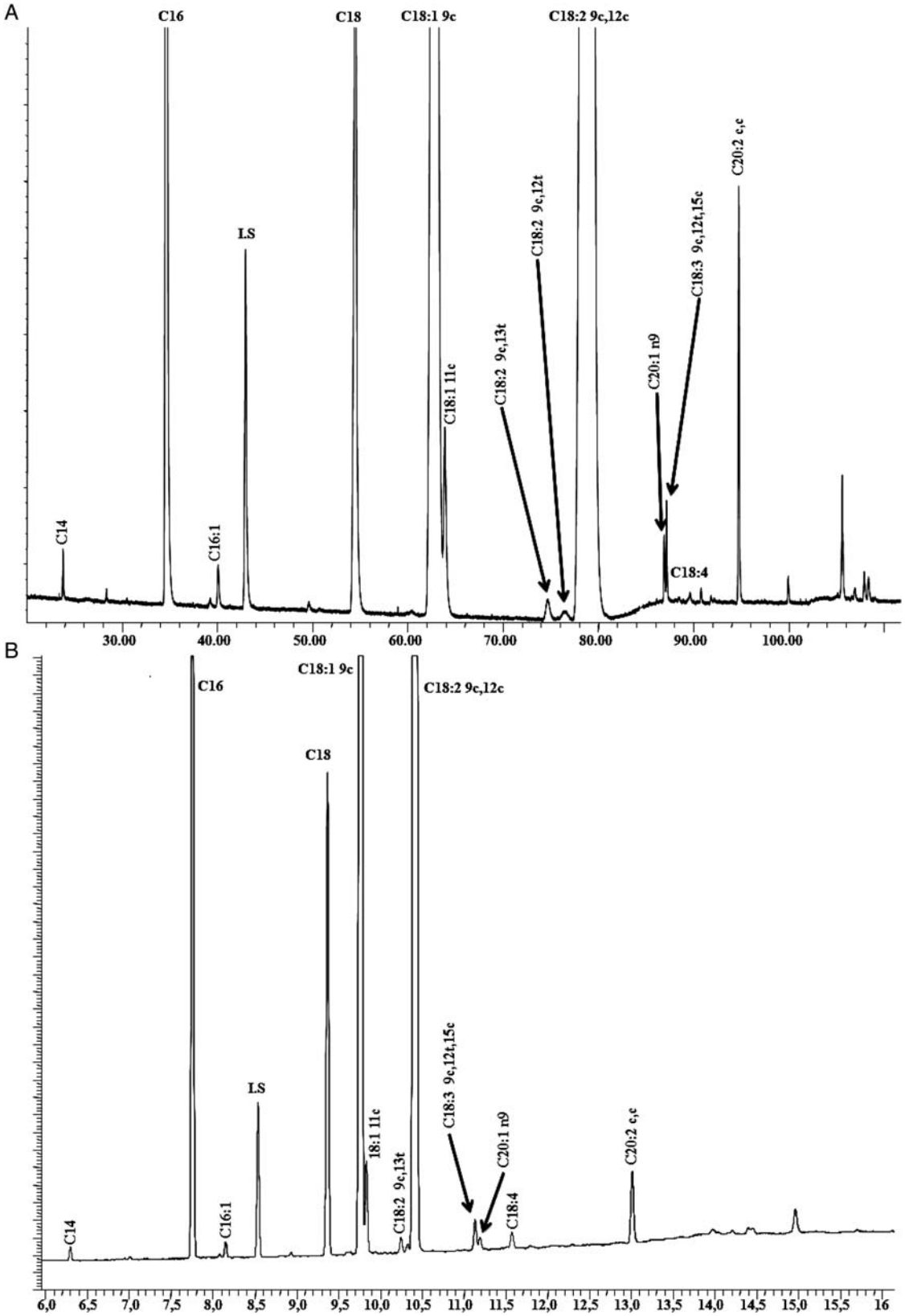


**Figure 3.** Fatty acid profile of sunflower oil sample: 100-m CP-Sil 88 column (A); 30-m VF23ms column (B).

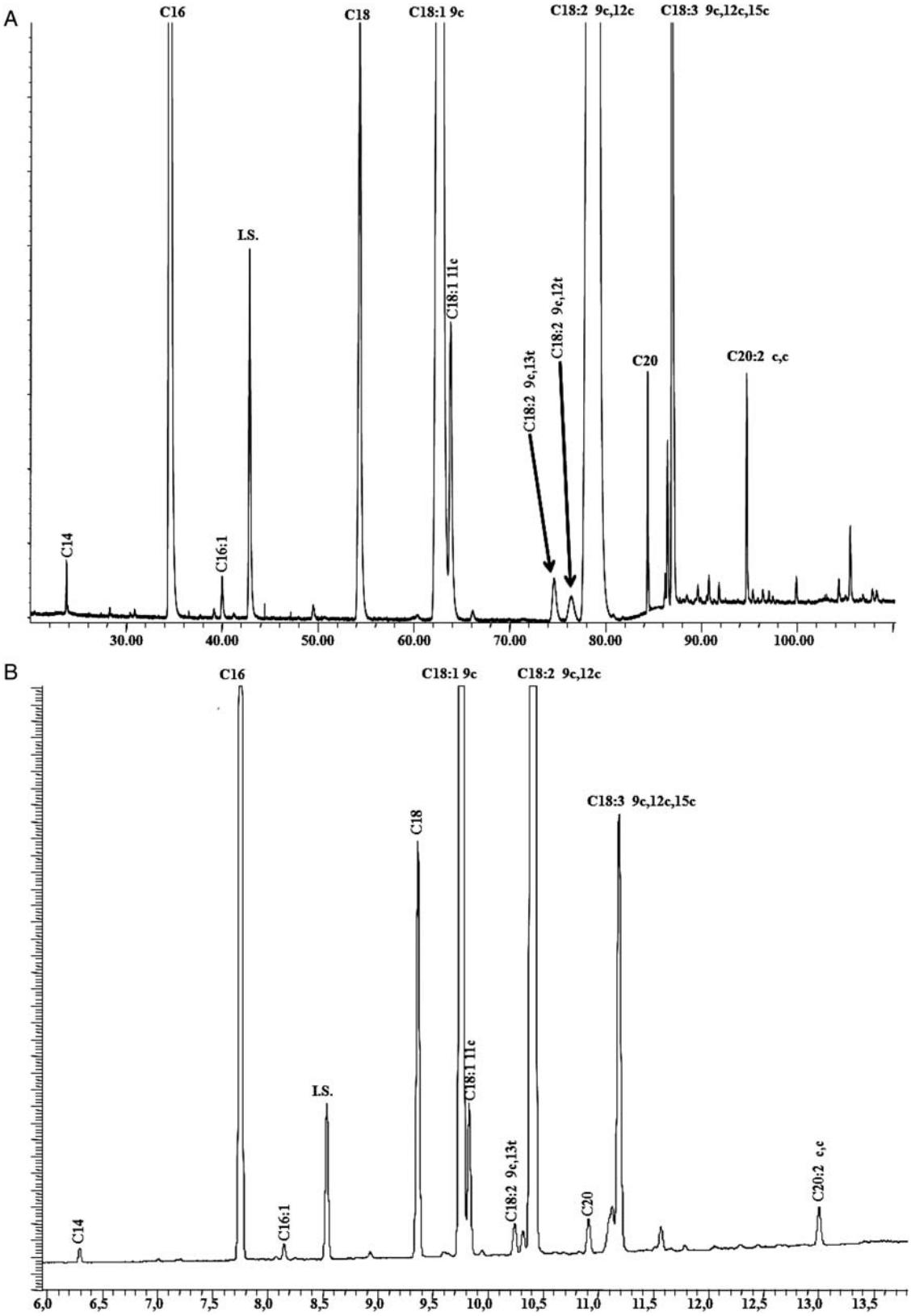


**Figure 4.** Fatty acid profile of soya oil sample: 100-m CP-Sil 88 column (A); 30-m VF23ms column (B).

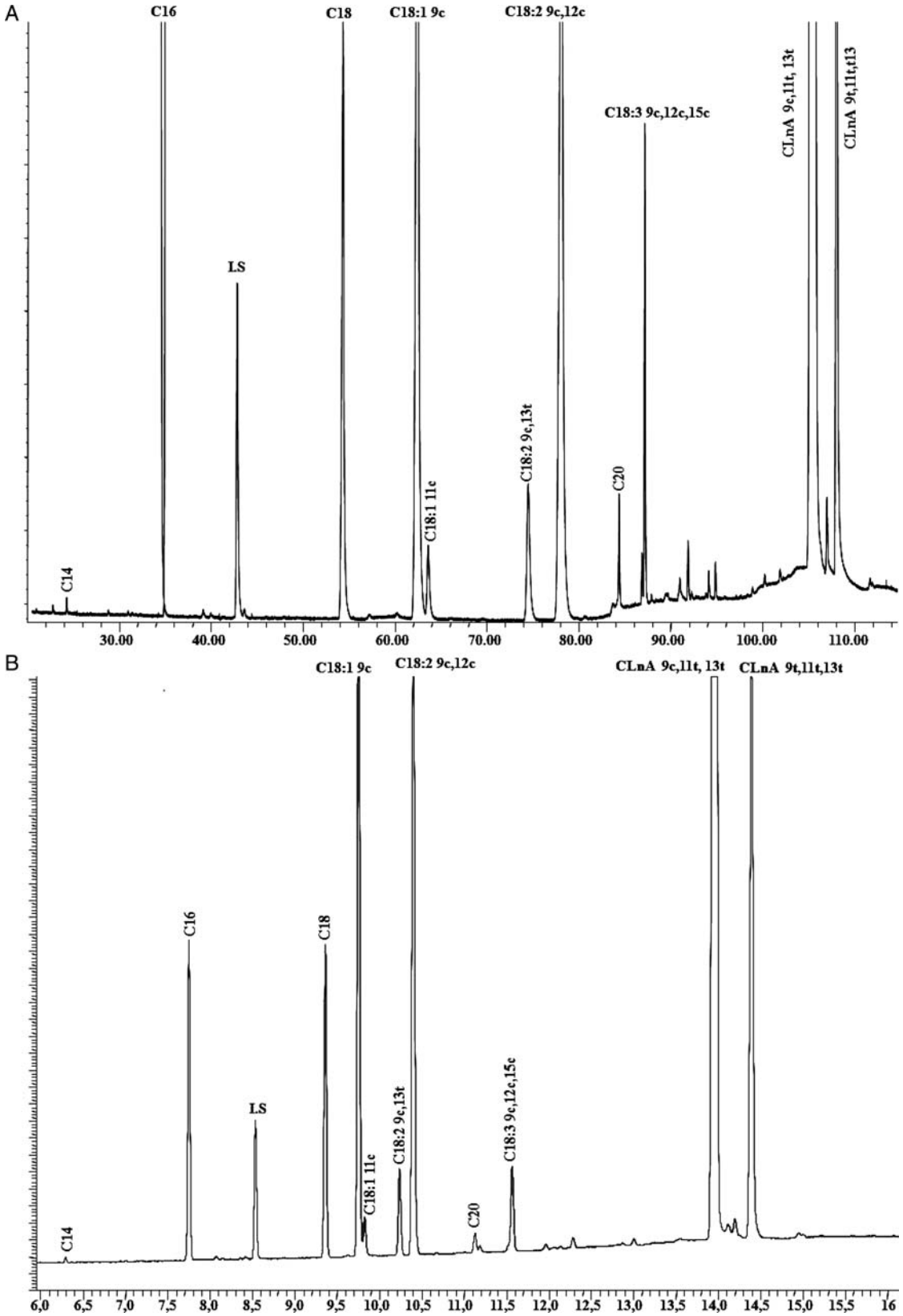


**Figure 5.** Fatty acid profile of tung oil sample: 100-m CP-Sil 88 column (A); 30-m VF23ms column (B).

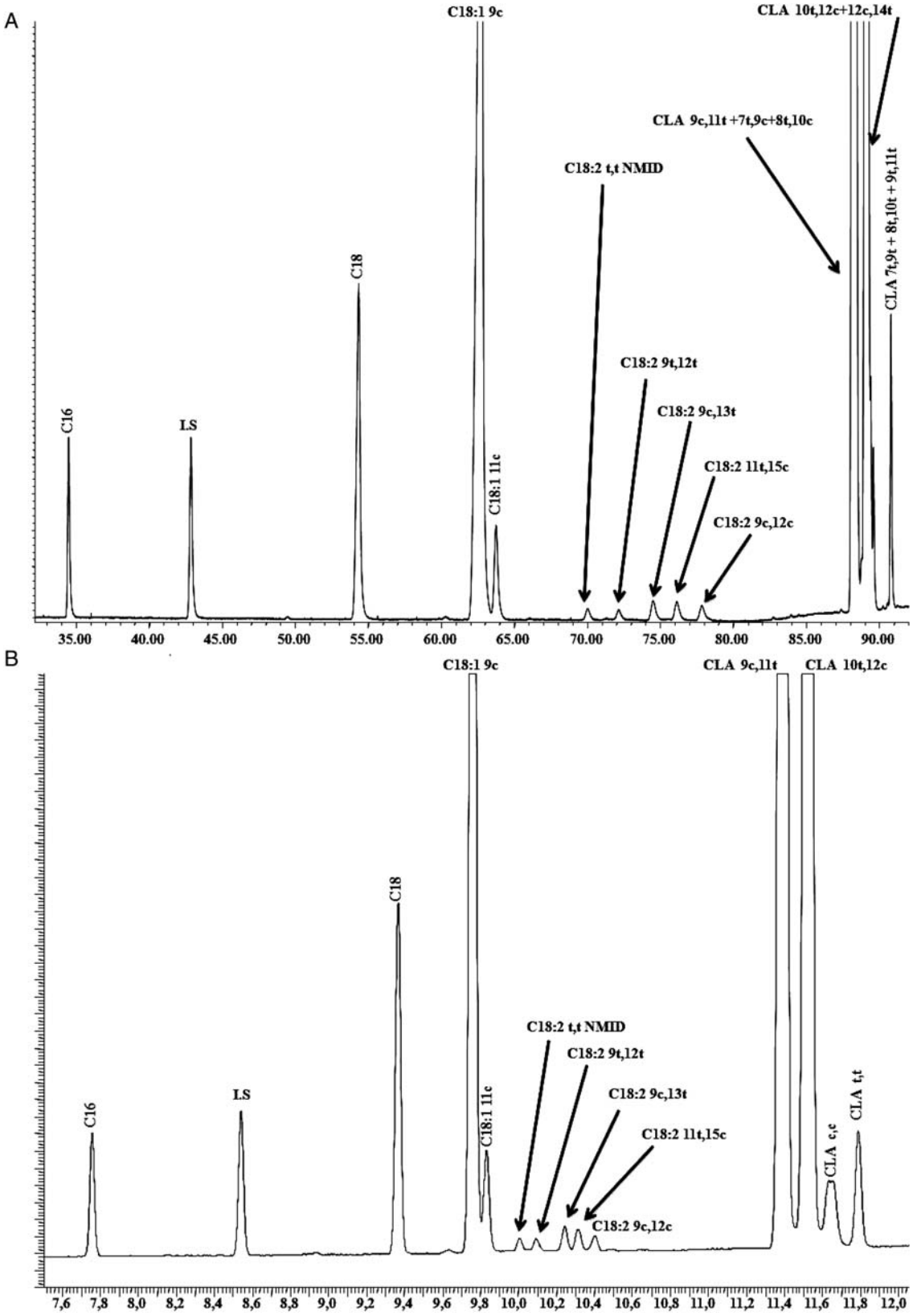


**Figure 6.** Fatty acid profile of tonalin oil sample: 100-m CP-Sil 88 column (A); 30-m VF23ms column (B).

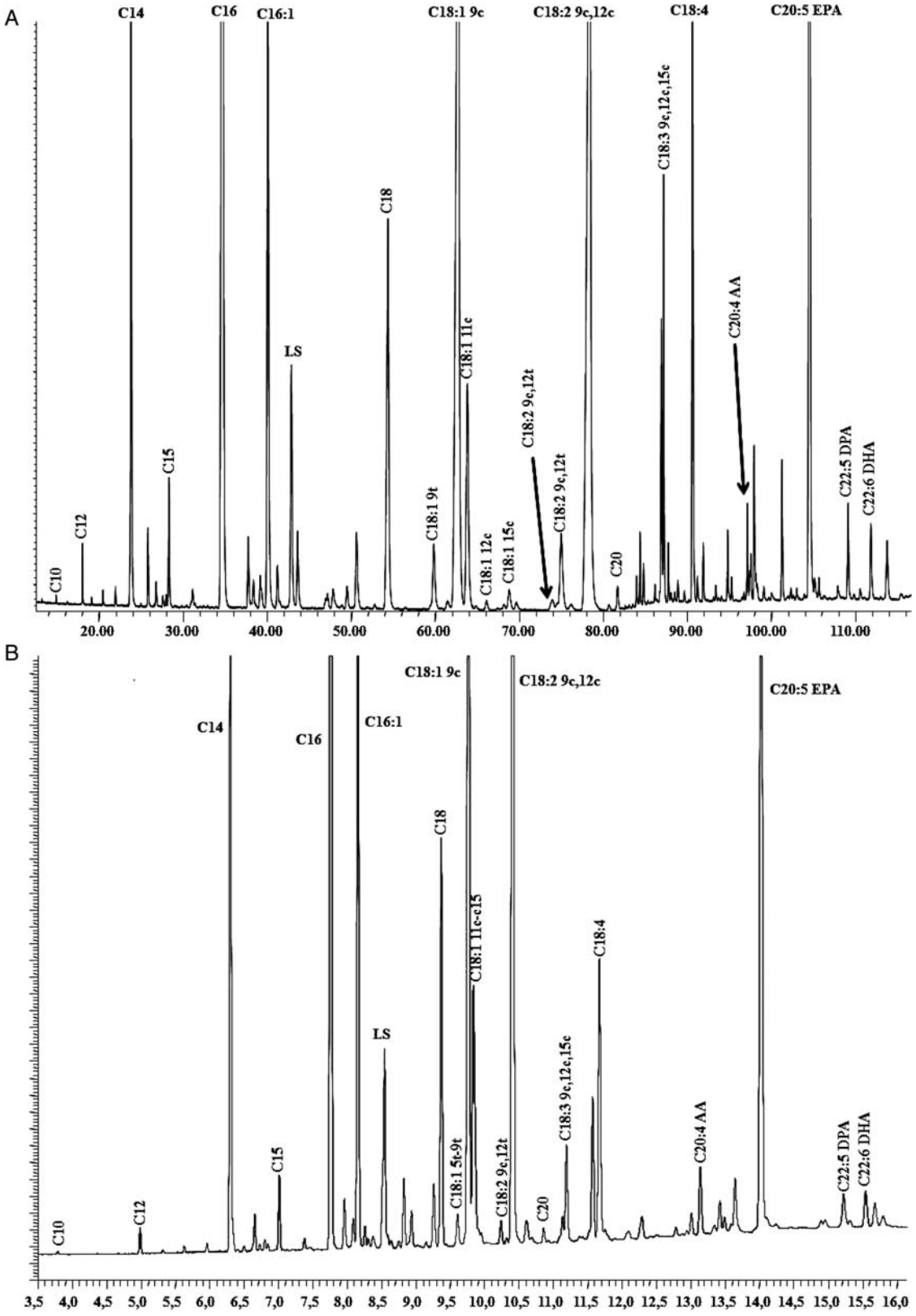


**Figure 7.** Fatty acid profile of fish oil sample: 100-m CP-Sil 88 column (A); 30-m VF23ms column (B).

involves the utilization of a 100-m SP-2560 capillary column and an isothermal program with 60 min of total run time (14). In the analysis of milk fat in which FA ranges from butyric acid (C4) to AA, in some cases with isomers, an isothermal program is not a proper solution. Further studies have compared the FA analysis according to the official method to that using the CP-Sil 88 (16). Chromatograms showed that both columns failed in the resolution of C18:1 6t-8t, C18:1 9t (elaidic acid), C18:1 10t and C18:1 11t from margarine samples. Other studies using a temperature program for the analysis of milk fat with both columns (total run: 114 and 111 min, respectively) reported that *trans*-octadecenoic acids eluted in one single peak with the SP-2560, while CP-Sil 88 achieved the total separation of these compounds (27). On the other hand, AOCS method Ce 1i-07 was designed only for the analysis of marine oils containing long chain fatty acids with a 30-m column (28). Ce 1j-07 was intended for the analysis of saturated and unsaturated fatty acids in ruminant fats, but the isomer quantification still needed be accomplished with extra analysis (14).

According to the results of our research work, the analysis of *trans*-octadecenoic acids in complex samples such as milk fat can be performed with the proposed column and method in total run times lower than those using a CP-Sil 88 and higher resolution than with an SP-2560. Even more, using both P0 and P1 programs, the elution of LA, LnA and CLA isomers and LCPUFA was possible.

### *Comparison of the quantitative results*

#### *Intra-assay results*

The FA composition from the analysis of the assayed samples (Tables I and II) with the VF23ms agreed with the data previously reported for milk fat (29), safflower, sunflower, soya (30), tonalin (23), tung (31) and fish oils (32, 33). As shown in the tables, milk fat was characterized by the presence of SFAs, primarily palmitic and stearic. Among MUFAs, oleic acid was the primary compound in all samples, showing values of 30.87% and 28.85% in sunflower and soya oils. The concentration of LA was 5.62% in tung oil, 1.72% in milk fat and 13.95% in fish oil; meanwhile, the amounts in safflower, soya and sunflower comprised from 46.45% to 68.83%., The higher value found for LnA was 4.66% in soya oil, the other values ranged from 0.12 to 1.21% for milk fat, safflower, sunflower, tung and fish oils, and

**Table I**
Fatty Acid Composition of Milk-Fat Sample (g FAME / 100 g fat) Analyzed with 100-m CP-SIL 88 Versus 30 m VF23m Columns*

| CP-Sil 88 | | | | | | | | VF23ms | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fatty acids | Rt (min) | Mean | RSD1 | Fatty acids | Rt (min) | Mean | RSD1 | Fatty acids | Rt (min) | Mean | RSD1 | RSD2 |
| C4 | 11.70 | 3.56 | 2.22 | C18:1 15c | 68.76 | 0.12 | 3.92 | C4 | 2.26 | 3.55 | 2.20 | 0.35 |
| C6 | 12.21 | 2.33 | 0.69 | C18:2 t,t NMID | 70.96 | 0.06 | 4.27 | C6 | 2.82 | 2.28 | 1.63 | 1.46 |
| C8 | 13.14 | 1.29 | 0.79 | C18:2 9t,12t | 71.77 | 0.16 | 1.80 | C8 | 3.76 | 1.19 | 0.91 | 5.87 |
| C10 | 14.85 | 2.82 | 0.85 | C18:2 9c,13t | 74.15 | 0.09 | 4.66 | C10 | 5.16 | 2.62 | 0.56 | 5.20 |
| C10:1 | 16.24 | 0.26 | 1.61 | C18:2 9c,12t | 75.17 | 0.04 | 9.98 | C10:1 | 5.75 | 0.29 | 1.30 | 6.07 |
| C12 | 18.00 | 3.58 | 0.57 | C18:2 11t,15c | 76.16 | 0.03 | 4.13 | C12 | 7.20 | 3.76 | 0.36 | 3.43 |
| C14 | 23.86 | 10.43 | 0.38 | C18:2 9t,12c | 76.69 | 0.07 | 5.05 | C14 | 10.08 | 10.28 | 0.37 | 1.08 |
| C15i | 25.84 | 0.26 | 0.41 | C18:2 9c,12c | 77.96 | 1.59 | 0.14 | C15i | 10.90 | 0.30 | 4.86 | 8.95 |
| C14:1t | 26.28 | 0.02 | 9.84 | C18:2 9c,15c | 79.23 | 0.08 | 7.30 | C14:1 | 11.02 | 1.09 | 0.73 | 6.49 |
| C15ai | 26.81 | 0.50 | 0.96 | C20 | 84.34 | 0.09 | 0.61 | C15ai | 11.24 | 0.56 | 0.91 | 8.06 |
| C14:1 | 27.61 | 1.00 | 0.55 | C18:3 n6 | 84.76 | 0.02 | 2.79 | C15 | 11.75 | 1.19 | 0.36 | 7.57 |
| C15 | 28.33 | 1.07 | 0.51 | C20:1 t | 86.20 | 0.02 | 10.21 | C16i | 12.66 | 0.18 | 5.53 | 1.15 |
| C16i | 31.14 | 0.18 | 2.34 | C18:3 9c,12c,15c | 86.88 | 0.54 | 9.08 | C16 | 13.62 | 27.34 | 0.20 | 1.61 |
| C16 | 34.88 | 27.97 | 0.14 | C20:1 n9 | 87.10 | 0.06 | 8.13 | C17i | 14.30 | 0.42 | 4.95 | 4.08 |
| C17i | 38.35 | 0.40 | 1.47 | C18:2 9c,11t + 7t,9c + 8t,10c | 88.02 | 0.92 | 3.73 | C16:1 | 14.50 | 1.62 | 1.13 | 0.27 |
| C17ai | 39.15 | 0.38 | 0.82 | C21 | 89.26 | 0.02 | 7.89 | C17ai | 14.90 | 0.42 | 2.80 | 7.80 |
| C16:1 | 40.04 | 1.63 | 0.16 | CLA 11c,13tt | 90.18 | 0.02 | 10.06 | C17 | 15.50 | 0.54 | 3.88 | 4.97 |
| C17 | 42.84 | 0.58 | 1.20 | CLA 13t,15t | 90.55 | 0.02 | 10.54 | C18 | 17.33 | 10.76 | 0.23 | 0.49 |
| C17:1c | 49.50 | 0.30 | 1.72 | CLA 12t,14t | 90.76 | 0.03 | 10.48 | C18:1 4t-9t | 17.65 | 0.43 | 4.49 | 4.29 |
| C18 | 54.68 | 10.83 | 0.12 | CLA 11t,13t | 91.22 | 0.01 | 10.21 | C18:1 10t | 17.68 | 0.54 | 5.80 | 10.17 |
| C18:1 5t | 57.48 | 0.04 | 5.21 | CLA 7t,9t + 8t,10t | 91.86 | 0.04 | 10.44 | C18:1 11t | 17.70 | 1.43 | 4.95 | 4.41 |
| C18:1 4t | 58.63 | 0.02 | 9.15 | C20:2 c,c | 93.11 | 0.03 | 2.69 | C18:1 9c | 17.81 | 23.29 | 0.13 | 5.59 |
| C18:1 6t-8t | 59.50 | 0.16 | 2.17 | C22 | 94.70 | 0.05 | 4.15 | C18:1 11c | 17.91 | 1.08 | 1.43 | 7.91 |
| C18:1 9t | 59.91 | 0.24 | 3.32 | C20:3 n6 | 95.20 | 0.11 | 4.01 | C18:1 12c | 17.99 | 0.40 | 1.82 | 3.65 |
| C18:1 10t | 60.39 | 0.47 | 1.90 | C20:4 AA | 97.89 | 0.19 | 4.98 | C18:1 13c + 14c | 18.13 | 0.47 | 1.60 | 1.38 |
| C18:1 11t | 60.83 | 1.34 | 1.03 | C20:3 n9 | 101.22 | 0.02 | 7.95 | C18:2 t,t | 18.25 | 0.18 | 5.59 | 8.42 |
| C18:1 12t-14t | 61.76 | 0.44 | 0.92 | C20:5 EPA | 104.37 | 0.04 | 6.79 | C18:2 c,t | 18.36 | 0.13 | 7.93 | 0.07 |
| C18:1 9c | 62.98 | 21.52 | 0.19 | C22:5 DPA | 105.55 | 0.03 | 3.97 | C18:2 t,c | 18.44 | 0.11 | 4.90 | 4.29 |
| C18:1 11c | 63.79 | 0.97 | 1.12 | C22:6 DHA | 118.91 | 0.05 | 3.20 | C18:2 9c,12c | 18.52 | 1.72 | 0.28 | 5.51 |
| C18:1 12c | 64.89 | 0.38 | 1.05 | SFA | | 66.37 | | C18:3 9c,12c,15c | 19.23 | 0.48 | 2.84 | 8.08 |
| C18:1 13c | 66.10 | 0.08 | 7.62 | MUFA | | 29.43 | | CLA 9c,11t | 19.38 | 0.88 | 1.91 | 3.06 |
| C18:1 16t + 14c | 67.74 | 0.36 | 2.44 | PUFA | | 4.20 | | CLA c,c + t,t | 19.51 | 0.13 | 5.23 | 5.58 |
| | | | | | | | | C20:3 | 20.38 | 0.12 | 3.09 | 4.00 |
| | | | | | | | | C20:4 AA | 20.60 | 0.22 | 2.75 | 7.91 |
| | | | | | | | | SFA | | 65.39 | | |
| | | | | | | | | MUFA | | 30.64 | | |
| | | | | | | | | PUFA | | 3.96 | | |

*Note: i: iso; ai: anteiso; t: *trans*; c: *cis*; NMID: non methylene interrupted diene; CLA: conjugated linoleic acid; AA: arachidonic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. RSD1 (relative standard deviation) calculated with means and standard deviations from data ($n=3$). RSD2 used in the inter-assay test calculated with the corresponding mean and standard deviation from CPSil-88 and VF23ms.

**Table II**
Fatty Acid Composition of Different Edible Oils (g FAME / 100 g Oil) Analyzed with 30-m VF23ms Column*

| | | Fish | | Safflower | | Sunflower | | Soya | | Tung | | Tonalin | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fatty acid | Rt (min) | Mean | RSD1 | Mean | RSD1 | Mean | RSD1 | Mean | RSD1 | Mean | RSD1 | Mean | RSD1 |
| C10 | 3.79 | 0.03 | 9.55 | | | | | | | | | | |
| C12 | 4.99 | 0.20 | 5.80 | 0.21 | 1.99 | | | | | | | | |
| C14 | 6.30 | 9.25 | 4.98 | 0.22 | 1.54 | 0.09 | 2.44 | 0.10 | 6.05 | 0.04 | 1.99 | | |
| C15 | 7.01 | 0.60 | 5.63 | 0.02 | 2.33 | 0.02 | 5.84 | | | | | | |
| C16 | 7.76 | 20.65 | 4.62 | 8.99 | 0.40 | 7.66 | 0.47 | 13.35 | 0.38 | 2.70 | 0.26 | 0.93 | 0.34 |
| C17iso | 7.96 | 0.60 | 3.57 | 0.06 | 2.69 | | | | | | | | |
| C16:1 | 8.16 | 6.45 | 4.53 | 0.10 | 0.63 | 0.12 | 0.49 | 0.12 | 1.67 | | | | |
| C17ai | 8.26 | 0.52 | 4.20 | | | | | | | | | | |
| C18 | 9.37 | 4.72 | 4.81 | 3.16 | 0.16 | 4.39 | 1.18 | 3.89 | 0.63 | 3.07 | 0.08 | 3.04 | 0.26 |
| C18:1 5t-9t | 9.61 | 1.22 | 5.66 | | | | | | | | | | |
| C18:1 10t | 9.63 | | | | | | | | | | | 0.04 | 4.38 |
| C18:1 9c | 9.76 | 18.03 | 4.67 | 15.50 | 0.07 | 30.87 | 0.86 | 28.85 | 0.97 | 7.63 | 0.23 | 14.32 | 0.16 |
| C18:1 11c-15c | 9.84 | 4.14 | 3.88 | 0.94 | 0.19 | 0.99 | 2.48 | 1.49 | 3.75 | 0.37 | 3.97 | 0.90 | 2.22 |
| C18:2 t,t NMID | 10.00 | | | | | | | | | | | 0.11 | 3.40 |
| C18:2 9t,12t | 10.09 | | | | | | | | | | | 0.09 | 6.53 |
| C18:2 9c,13t | 10.23 | 0.21 | 7.02 | 0.75 | 0.16 | 0.12 | 9.29 | 0.24 | 2.66 | 0.68 | 0.29 | 0.17 | 1.55 |
| C18:2 9c,12t | 10.24 | 0.22 | 5.16 | 0.67 | 0.32 | 0.06 | 1.08 | 0.19 | 4.76 | | | | |
| C18:2 11t,15c | 10.31 | | | | | | | | | | | 0.16 | 1.12 |
| C18:2 9c,12c | 10.41 | 13.95 | 4.85 | 68.83 | 0.01 | 54.75 | 0.32 | 46.45 | 0.65 | 5.62 | 0.13 | 0.12 | 1.63 |
| C20 | 10.86 | 0.21 | 7.02 | | | | | 0.28 | 1.40 | | | | |
| C18:3 9c,12c,15c | 11.19 | 1.21 | 5.98 | 0.12 | 3.54 | 0.11 | 2.01 | 4.66 | 1.20 | 0.10 | 6.13 | | |
| C20:1 n9 | 11.20 | | | | | 0.16 | 0.68 | | | | | | |
| CLA 9c,11t | 11.39 | | | | | | | | | | | 39.12 | 0.11 |
| CLA 10t,12c | 11.53 | | | | | | | | | | | 38.95 | 0.08 |
| CLA c,c | 11.63 | | | | | | | | | | | 1.07 | 0.67 |
| C18:4 | 11.67 | 3.25 | 4.27 | 0.17 | 2.86 | | | | | | | | |
| CLA t,t | 11.79 | | | | | | | | | | | 0.97 | 0.72 |
| C20:2 c,c | 13.01 | | | 0.25 | 0.41 | 0.65 | 1.40 | 0.37 | 1.09 | | | | |
| C20:4 AA | 13.14 | 0.78 | 3.99 | | | | | | | | | | |
| CLnA 9c,11t,13t | 13.96 | | | | | | | | | 73.32 | 0.09 | | |
| C20:5 EPA | 14.02 | 11.39 | 4.45 | | | | | | | | | | |
| CLnA 9t,11t,13t | 14.39 | | | | | | | | | 6.47 | 0.41 | | |
| C22:5 DPA | 15.54 | 0.97 | 4.73 | | | | | | | | | | |
| C22:6 DHA | 15.68 | 1.40 | 0.44 | | | | | | | | | | |
| SFA | | 36.77 | | 12.67 | | 12.16 | | 17.63 | | 5.81 | | 3.98 | |
| MUFA | | 29.84 | | 16.54 | | 32.14 | | 30.46 | | 8.01 | | 15.26 | |
| PUFA | | 33.38 | | 70.79 | | 55.69 | | 51.91 | | 86.18 | | 80.76 | |

*Note: i: iso; ai: anteiso; t: *trans*; c: *cis*; NMID: non methylene interrupted diene; AA: arachidonic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; RSD1 (relative standard deviation) calculated with means and standard deviations from data ($n = 3$).

it was not detected in tonalin oil. As expected, the presence of CLA was only detected in milk fat and tonalin oil. In fish oil, very long PUFAs such as EPA, DPA and DHA were quantified (11.39%, 0.97% and 1.40%).

The results of the intra-assay analysis are shown in Tables I and II. The FA values obtained by applying the P0 program to milk fat samples and the P1 program to vegetable and fish oils were below the allowance limit (10%) proposed by Horwitz (21) and denoted a high level of reproducibility. Furthermore, using the VF23ms column, the total analysis time required for the elution of all peaks appearing in milk fat, vegetable oils and fish oils was approximately 20 min.

### *Inter-assay results*

The RF obtained from the analysis of the standard CMR-164 with known FA concentration in the VF23ms column were close to unity, which assures the accuracy of the quantification. The RSDs were calculated from the concentrations achieved with both VF23ms and CP-Sil 88 columns in the analysis of milk (Table I). Butyric (C4), caproic (C6) and caprylic (C8) acids are characteristic compounds of milk fat. Because they are extremely volatile in the form of FAME, their analysis is a critical issue in these samples. The comparison of the concentrations of those fatty acids displayed by VF23ms versus CP-Sil 88 showed RSD values of 0.35, 1.46 and 5.87, respectively. Myristic, palmitic and stearic acids were the major SFA in milk fat; their RSDs were below 1.5%. Oleic, LA and LnA also showed good results (5.58, 5.51 and 8.07, respectively), as did other minor compounds such as the *trans*-octadecenoic fatty acids and CLA isomers.

These results proved the accuracy of the proposed methods in the analysis of FAs from SCFAs to LCFAs, primarily in essential omega-6 and omega-3, *trans*-octadecnoic FA and CLA in edible fats and oils.

## Conclusions

The present study tested two temperature programs to be used in a 30-m-long VF23ms capillary column as routine methods in the analysis of fatty acids in milk fat and fish and vegetable oils. In the assayed conditions, run times were less than 20 min; short chain FAs and major SFAs, oleic, LA and LnA, *trans*-octadeceoic, CLA and CLnA isomers and other omega-6 and omega-3 fatty acids were identified and quantified. When the obtained concentrations were compared with theoretical contents and with those from a high-resolution 100-m CP-Sil 88 column, the results showed good accuracy and feasibility.

The results in the present study confirm that a 30-m VF23ms column together with the P0 and P1 temperature programs can be used as a routine method for analysis of fatty acids of edible fats and oils.


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