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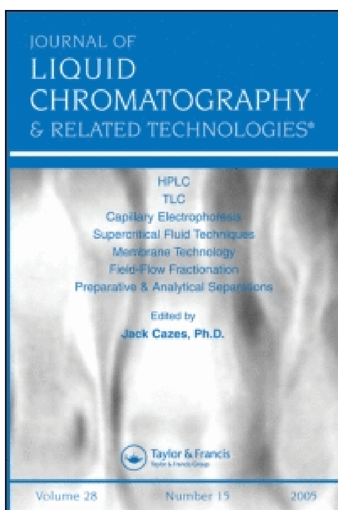

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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S. Héron[a]; E. Lesellier[a]; A. Tchapla[a]
[a] LETIAM - IUT d'Orsay Plateau du moulon, Orsay, BP, Cedex, France

To cite this Article Héron, S. , Lesellier, E. and Tchapla, A.(1995) 'Analysis of Triacylglycerols of Borage Oil by RPLC Identification by Coinjection', Journal of Liquid Chromatography & Related Technologies, 18: 3, 599 — 611
To link to this Article: DOI: 10.1080/10826079508009260
URL: http://dx.doi.org/10.1080/10826079508009260

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JOURNAL OF LIQUID CHROMATOGRAPHY, 18(3), 599-611 (1995)

ANALYSIS OF TRIACYLGLYCEROLS OF BORAGE OIL BY RPLC IDENTIFICATION BY COINJECTION

S. HÉRON*, E. LESELLIER, AND A. TCHAPLA

LETIAM - IUT d'Orsay
Plateau du moulon BP 127
91403 Orsay Cedex, France

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ABSTRACT

Borage oil is an interesting oil because it is rich in gamma linolenic acid (γLn 18:3 Z6, Z9, Z12). However, since triacylglycerol standards with γLn chains do not exist , there is a problem identifying these particular triacylglycerols. Because the hyphenated techniques (GC-MS, LC-MS) do not give sufficient resolution for unequivocal identification, an easier methodology is described here. It consists of using another oil of known composition as a standard mixture. Use of this method is shown for the assignment of triacylglycerols in borage oil.

INTRODUCTION

Oils found in biological fluids or in foods are complex mixtures which have always been studied by the analytical chemist (1). The challenge of separation for such mixtures is different depending on the class of compounds of interest. Thus, the separation techniques employed are very different if information is desired about one

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class with regard to another (i.e. tocopherols, sterols, carotenoids, triacylglycerols) or about one or more specific compound.

One of the most important classes are the triacylglycerols, the main constituents of oils and fats. Many papers have already been published on this class, proposing different chromatographies, such as capillary gas chromatography (2-4), planar chromatography (5-7), argentation chromatography (8), or reversed phase liquid chromatography (9-11), with treatment or not of the sample.

Whatever the technique used, after obtaining the separation from direct injection of a given oil or fat, the last step consists of identifying the triacylglycerols. Due to the complexity of all the chromatograms, an alternative analytical method consists of preseparation and isolation of different fractions of triacylglycerols as a function of their total unsaturation number. In subsequent stage, each fraction can be analysed and characterized (in terms of triacylglycerol identification either by NPLC, RPLC or capillary gas chromatography). Many methods have been employed to answer this question, such as injection of triacylglycerol standards, use of predictive diagrams (12-13), hyphenated techniques (gas or liquid chromatography coupled with mass spectrometer detector) (3,14). The characterization of oils and fats after a treatment of the sample such as a transesterification of triacylglycerols into methyl esters has also been used (1,15,16). However, these methods do not allow for the easy identification of triacylglycerols with similar structures (i.e., same total number of carbon atoms and same total number of double bonds in each chain, but differing in the distribution along the chain) because their retention times are close together.

The method for the identification that has been developed in our laboratory consists in enriching the unknown oil with an oil of known composition. The comparison of the chromatograms of this mixture and of the pure oils allows a more accurate identification.

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MATERIALS AND METHODS

Samples

The oils have been bought in supermarkets or kindly donated by the dermatological or cosmetological research center of Pierre Fabre (Gigoulet, France) or the Christian Dior (Orléans, France) and used without further purification. The injection solvent was acetone.

Solvents

Acetonitrile and methylene chloride were HPLC grade (Merck, Darmstadt, Germany; Carlo-Erba, Milan, Italy) and filtered through a 0,5 μm Millipore filter (Whatman, Hillsboro, OR, USA).

Apparatus

The chromatographic system consisted of a Model 1050 pump (Hewlett Packard, Palo-Alto, CA, USA), a model 7125 injection valve with a 20 μl loop (Rheodyne, Cotali, CA, USA) and a Model Sedex 45 light-scattering detector (Sedere, Alfortville, France).The column temperature was controlled using a Croco-cil oven (Cluzeau, Sainte-Foy-la-Grande, France) thermostated with water by means of a Model UCF10 cryostat with a precision of 0.1°C (Julabo, Seelbach, Germany, supplied by Touzart et Matignon, Vitry-sur-Seine, France). The mobile phase flow-rate was 1 ml / min. A Brownlee Spheri 5 ODS (5μm) 250 x 4,6 mm column was used (Perkin Elmer, Applied Biosystems Division, Foster City, CA, USA).

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RESULTS AND DISCUSSION

Reversed phase liquid chromatography (RPLC) was the separation technique employed. The first step taken was to find simple conditions which would allow the separation of critical pairs of triacylglycerols (i.e. with the same total number of carbon atoms and the same total number of double bonds, differing in the distribution of these unsaturations sites within the three chains). To solve this problem, a systematic study of standards of homogenous triacylglycerol mixture on different stationary phases and with mobile phases of different nature and composition (11,17). The results of this study allowed us to propose optimum conditions for the separation of triacylglycerols with only one column and a binary mobile phase which was suitable for forty oils and fats (18-19).

The composition of many of the analyzed oils were already well known. However, other oils were also studied which have never been characterized. Few standards of triacylglycerols are commercially available, so their use gives little information for the identification of other unidentified triacylglycerols. In order to get further information, the use of hyphenated techniques such as gas chromatography or

liquid chromatography coupled with mass spectrometry is an alternative. It necessitates working with both electron impact as well as chemical ionization mode instrumentation. Initially, a GC-MS system was used in our laboratory: however, after identification of the triacylglycerols of an oil by this technique, each peak had to be matched with the corresponding peak in RPLC. But there are two limitations to this method of identification. First, the retention order is not the same in GC as in RPLC. Second, highly unsaturated compounds give very large peaks. This leads to poor chromatographic separation and a loss of information in MS. A priori, these two restrictions could be avoided by using LC-MS systems. However, some problems remain, particularly when the chromatogram has two closely spaced peaks with very different peak heights. In this case, the identification of minor peaks could be either very difficult or impossible. Thus, this latter technique can only give suitable identification of the well separated peaks for any analysed oil.

This is the case of the borage oil. It is an unusual oil because of its high content in gamma-linolenic acid (γLn 18:3 Z6, Z9, Z12) (1,20,21). Since commercial standard triacylglycerols with γLn chains do not exist , there is a problem identifying triacylglycerols which contain at least one of this residue. One possible method for analysing the triacylglycerol residues consists of using statistical calculations. However, this oil cannot be indexed simply by correlation of area percentage of peaks and this statistical manipulation: although its fatty acid composition is known, it is not possible to deduce its corresponding triacylglycerol composition by such a calculation. Indeed, pure statistical treatments lead to the following results : the more abundant a fatty acid, the more abundant the corresponding triacylglycerol, which is not always experimentally observed (22). The resulting calculations still could lead to erroneous conclusions as to the fatty acid composition. If the fatty acid composition of borage oil is considered, the triacylglycerols present could be various combinations of γ linolenic acid and linolenic acid (such as LnLnγLn and γLnγLnLn for example or OγLnγLn and OLnLn) independent of their relative amounts. This problem occurs because even under the best possible experimental separation conditions (11), these particular triacylglycerols are not totally resolved. Thus a second possible alternative to the identification of the triacylglycerol residues might be through the use of a MS detector. But, in this case, LC-MS could not distinguish between these two different triacylglycerols of each critical pair of peaks due to the similarity of the mass spectra of each.

Consequently, another strategy has been used to identify the major components of borage oil (Figure 1 whose comments about identification will be given later). This was done by spiking the sample oil with another oil of known composition. This method was previously used successfully in a similar situation to identify isomeric triacylglycerols

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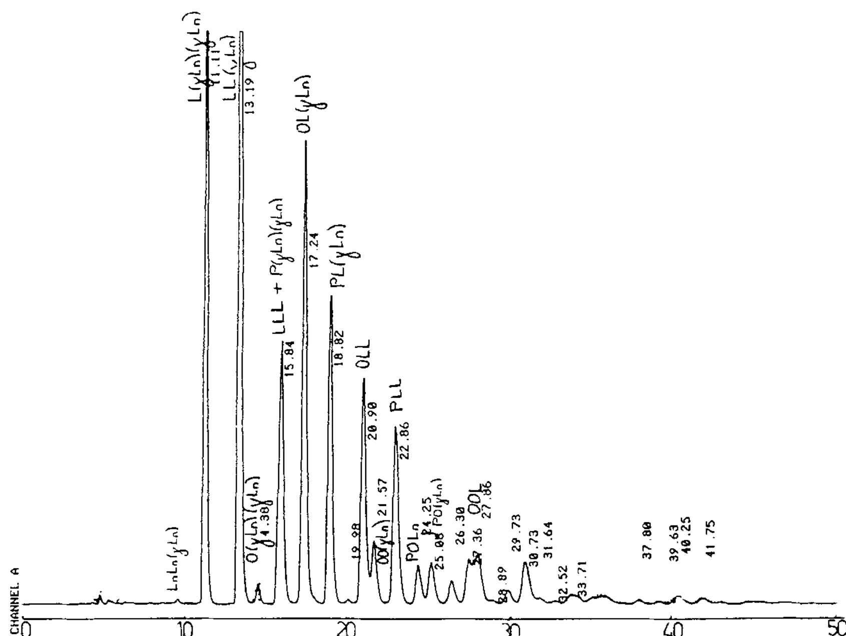

Figure 1: Chromatogram of the borage oil.
Column: Brownlee Spheri 5 ODS
Mobile phase: MeCN / CH_2Cl_2 68 / 32
Temperature: 21°C
P : palmitic acid (16:0); O : oleic acid (18:1, Z9); L : linoleic acid (18:2, Z9, Z12), Ln : linolenic acid (18:3, Z9, Z12, Z15); γLn : gamma linolenic acid (18:3, Z6, Z9, Z12).

with residues possessing cis and trans ethylenic double bonds (23). In our case, major problem occured during the choice of any oils or fats to use. Black current stone oil is one which also contains a significant content of γLn fatty acid (1,24,25). Thus, black current stone oil can be considered as a standard triacylglycerols with γLn residues similar to those found in borage oil (Figure 2). As the black current stone oil also has a large amount of Ln fatty acid, which is not the case with the borage oil, linseed oil which *is rich in* Ln but is free of γLn was used to distinguish triacylglycerols which possess at least a Ln residue (1).

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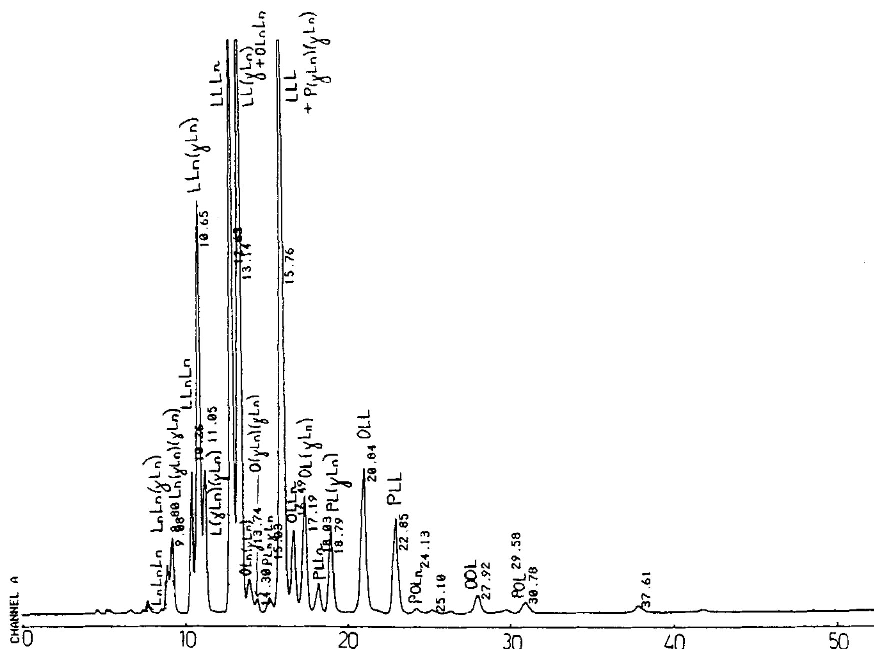

Figure 2: Chromatogram of the black current stone oil. For conditions, see Figure 1.

When the three chromatograms obtained from the previous described conditions (11,18) were compared, all the retention times were similar so that assignment of the peaks of the borage oil remained difficult. In addition, over several analyses, small shifts in the retention time of a given compound could be observed . To avoid this problem, mixtures of the oils (black current stone oil and linseed oil, black current stone oil and borage oil, linseed oil and borage oil) were made. The comparison of the respective heights of each peak of the mixtures with those of the pure non enriched oils allowed assignment of each peak to the correct triacylglycerol.

Identification of the borage triacylglycerols containing Ln and L fatty acids.

The first mixture studied was that of the two known oils taken as references, i.e. linseed oil and black current stone oil (Figure 4). The comparison of the fingerprint of each pure oil allowed confirmation of the peaks corresponding to triacylglycerols having

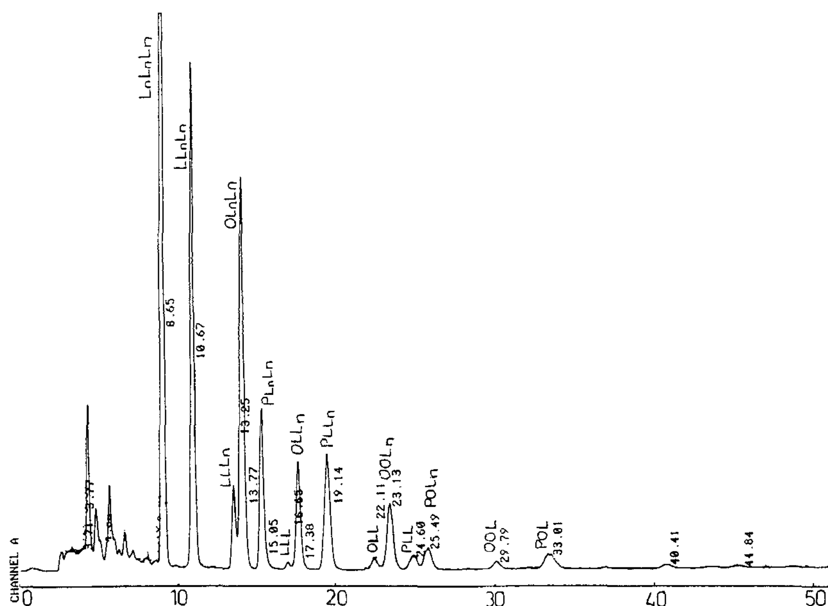

Figure 3: Chromatogram of the linseed oil.
For conditions, see Figure 1.

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the Ln fatty acid without the γLn fatty acid. Thus, in the chromatogram of the mixture (Figure 4), all the peaks whose intensity has increased in comparison with those in the chromatogram of the pure black current stone oil (Figure 2) are triacylglycerols which are found in the linseed oil. They are marked by an arrow on the chromatogram.

The chromatogram corresponding to a mixture (borage and linseed oils) is shown in Figure 5. The comparison with the non-enriched oils (Figure 1 and 3) allowed the conclusion that:

- The peaks to 8.7, 10.68, 13.25 and 15.05 min are due respectively due to the triacylglycerols LnLnLn, LLnLn, LLLn and PLnLn. They are not present in the pure borage oil.

- The intensities of the peaks at 16.61, 22.09 and 24.23 min have increased in comparison with those in the chromatogram of linseed oil (Figure 3). So, these peaks correspond respectively to LLL, OLL and PLL present in the two oils.

- Lastly, the peaks at 11.55, 18.12 and 19.85 min are in the pure borage oil but not in the linseed oil. They are identified later in this article.

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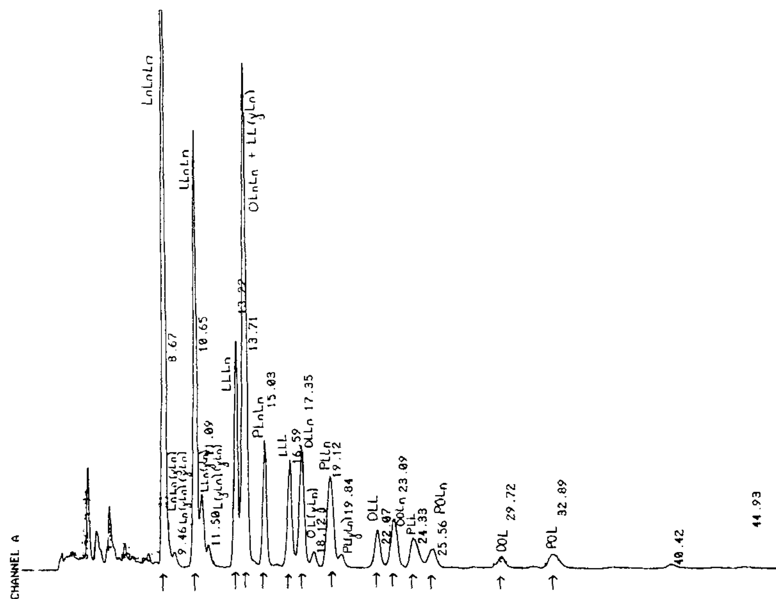

Figure 4: Chromatogram of the linseed oil enriched by black current stone oil.
For conditions, see Figure 1.

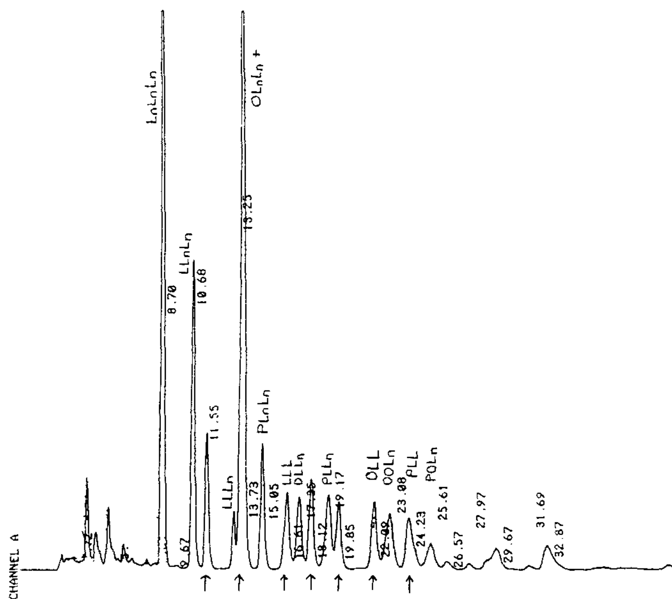

Figure 5: Chromatogram of the linseed oil enriched by borage oil .
For conditions, see Figure 1.

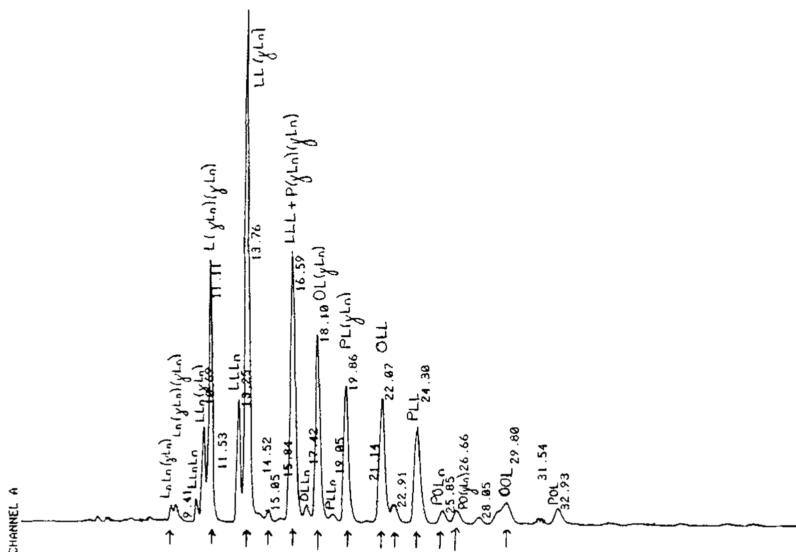

Figure 6: Chromatogram of the black current stone oil enriched by borage oil For conditions, see Figure 1.

- The intensity of the peak at 13.73 min has also increased in comparison with those in the chromatogram in Figure 3. So, one might be lead to believe that the triacylglycerol OLnLn is one of the compounds of borage oil. However, this peak is one of the most important in the chromatogram and it is known that there is little Ln fatty acid in borage oil. This did not seem correct. A reexamination of the chromatogram of mixture of black current stone oil and linseed oil (Figure 4) shows that OLnLn has the same retention time as LLγLn. Thus, it is probably this last triacylglycerol which could be in the borage oil, as it will be proven with the next mixture.

Identification of triacylglycerols containing γLn fatty acid.

The chromatogram of the mixture of borage and black current stone oil is given in Figure 6. The comparison with the chromatogram of black current stone oil (Figure 2) indicates that the peaks at 11.11, 13.76, 18.10 and 19.86 min are respectively due to LγLnγLn, LLγLn, OLγLn and PLγLn are also found in the borage oil.

In order to confirm the identifications, we have drawn the diagram of retention log k' versus the number of double bonds (DB) in the triacylglycerols (12,13). Goiffon et al have shown that the substitution of one residue by another has the same effect whatever the triacylglycerol, so for the four following triacylglycerols XXX- XXY-

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XYY-YYY, log k' vs DB must be on a straight line. If we consider the evolution of log k' values in triacylglycerols (or homologues), the log k' vs the number of carbon atoms (n_C) are not always perfect straight lines (26). However, in a small range of n_C values this non linearity does not lead to the non validity of the Goiffon diagram. Thus, for the three following triacylglycerols classes [PLL-PLγLn-PγLnγLn], [LLL, LLγLn-LγLnγLn], [OLL-OLγLn-OγLnγLn] composed by solutes whose identification was made by the above described spiking method, the log k' vs DB plots are straight lines, confirming the assignments.

CONCLUSION

The identification of triacylglycerols in an oil by its enrichment with other oils of known composition is an efficient and accurate method. As for any identification with a standard, the assignment of a peak of an unknown based on the same retention time compared to those of the chosen standard does not lead to unambiguous assignments. To confirm an identification it is imperative to proceed to some verification such as a change of experimental conditions or the examination of the chromatograms gained from the use of multiple standards.

The choice of reference oils must be governed by the following considerations : as the qualitative composition in fatty acids is directly connected to the composition of the triacylglycerol, the knowledge of fatty acid composition of any non identified oil is needed to permit the correct selection of the reference oil which possesses the wanted triacylglycerols necessary to help in the identification of the unknown oil. Moreover, this also allows the unambiguous elimination of some possible triacylglycerols by absence of their corresponding peak.

By using this spiking technique, the correct assignment of triacylglycerols to the main components of borage oil has been possible. The results are in agreement with the assignment proposed by Aitzetmüller et al (25) with a short wavelength UV detection, on columns giving different selectivities. Thus, the information obtained after analysis of mixtures allowed the assignment of the peaks seen in the borage oil chromatogram. The mixture with the black current stone oil allowed the identification of most of the triacylglycerols containing γLn fatty acid. This mixture alone does not allow the unambiguous identification of all the compounds. To complete the assignment, it is also necessary to examine the mixture containing linseed oil which showed the presence of triacylglycerols such as LLL, OLL or POL. This methodology was extended to confirm the identification of triacylglycerols constituting some forty oils we have analysed.

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ACKNOWLEDGMENTS

The authors thank Dr. F. Rabel (EM Separations Technology, Gibbstown, NJ, USA) for his comments and corrections throughout this study.

REFERENCES

1- A. Karleskind, Manuel des corps gras, Technique et Documentation, Inc., Lavoisier, Paris, 1992

2- E. Geeraert, Chromatography of lipids in biomedical research and clinical diagnosis, A. Kuksis, ed., J. Chromatogr. Library, Elsevier, Inc., Amstedam, vol. 37, 1987,
pp 48-75

3- W.W. Christie, Gas chromatography and lipids - A practical guide, the oily press, Inc., Ayr, Scotland, 1989

4- R.G. Ackman, Application of gas liquid chromatography to lipid separation and analysis: Qualitative and quantitative analysis in analyses of fats, oils and lipoproteins, Edward G. Perkins, ed., Americain oil chemists' Society, Inc., Champain, Illinois, 1991

5- R.G. Ackman, Flame ionization detection applied to thin layer chromatography or coated quartz rods, J.M. Lowenstein, ed., Methods in Enzymology, Part D, Lipids, Academic Press, Inc., New-York, vol 72, 1981, pp 205-252.

6- M. Ranny, Thin Layer Chromatography with flame ionization detection, D. Reidel, Amsterdam, 1987

7- R.E. Kaiser, Planar Chromatography, vol. 1, Hüthig, Inc., Heidelberg, 1986

8- B. Nikolova-Damyanova, Advances in Lipid Methodology - One, W.W. Christie, ed., Oily Press, Inc., Ayr, 1992, pp 181-237

9- R.J. Hamilton, J.B. Rossell, Analysis of oils and fats, Elsevier, Inc., 1986, pp 286-290

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10- L.J.R. Barron, G. Santa-Maria, Chromatographia, 23, 209-214, 1987

11- S. Héron, A. Tchapla, Analusis, 22, 114-126, 1994

12- J.P. Goiffon, C. Reminiac, M. Olle, Rev. Fr. Corps Gras, 28, 167-170, 1981

13- J.P. Goiffon, C. Reminiac, D. Furon, Rev. Fr. Corps Gras, 28, 199-207, 1981

14- A. Kuksis, L. Marai, J.J. Myher, S. Pind, Chromatography of lipids in biomedical research and clinical diagnosis, A. Kuksis, ed., J. Chromatogr. Library, Elsevier, Inc., Amsterdam, vol. 37, 1987, pp 403-440

15- S. Hudiyono, H. Adenier, H. Chavéron, Rev. Fr. Corps Gras, 40, 131-141, 1993

16- T.G. Toschi, W.W. Christie, L.S. Conte, HRC, 16, 725-729, 1993

17- S. Héron, A. Tchapla, New Trends in Lipid and Lipoprotein Analysis, EG Perkins and JL Sébédio, ed., Amer. Oil Chem. Soc., Press Pub, Champaign, IL, in press

18- S.Héron, A. Tchapla, Rev. Fr. Corps Gras, in press

19- S. Héron, A. Tchapla, Fingerprints of triacylglycerols from oils and fats by HPLC isocratic elution and evaporative light scattering detection ELSD SEDEX 45, Sedere, Alfortville, France, 1994

20- J.P. Blond, G. Durand, L. Ulmann, M.P. Lonjaret, J.P. Poisson, J. Bezard, G. Pascal, Actes du congrès international "Chevreul" pour l'étude des corps gras, Congrès Eurolipid, Association Française pour l'étude des corps gras, 1989, pp 694-701

21- K. Coupland, N.A. Langley, Actes du congrès international "Chevreul" pour l'étude des corps gras, Congrès Eurolipid, Association Française pour l'étude des corps gras, 1989, pp 1357-1363

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22- M.A.M. Zeitoun, W.E. Neff, E. Selke, T.L. Mounts, J. Liq. Chromatogr., 14, 2685-2698, 1991

23- P. Laakso, H. Kallio, J. Amer. Oil Chem. Soc., 70, 1161-1171, 1993

24- J.L. Perrin, A. Prévot, H. Traitler, U. Bracco, Rev. Fr. Corps Gras, 34, 221-223, 1987

25- K. Aitzetmüller, M. Grönheim, HRC, 15, 219-226, 1992

26- A. Tchapla, S. Héron, E. Lesellier, H. Colin, J. Chromatogr. A, 656, 81-112, 1993

Received: April 28, 1994
Accepted: August 29, 1994

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