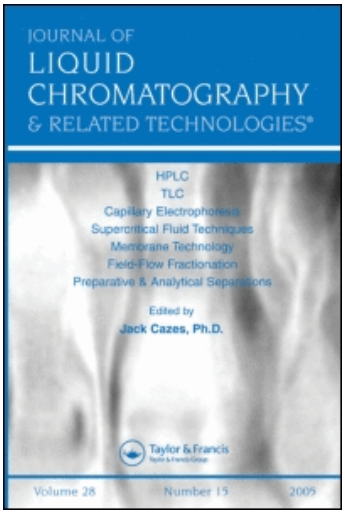
This article was downloaded by:
On: *25 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



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

Separation of Isomeric Triacylglycerols by Silver Ion Thin-Layer Chromatography

B. Nekolova-Damyanova[a]; D. Chobanov[a]; S. Dimov[a]
[a] Laboratory of Lipid Chemistry, Institute of Organic Chemistry with Center of Phytochemistry Bulgarian Academy of Sciences, Sofia, Bulgaria

To cite this Article Nekolova-Damyanova, B. , Chobanov, D. and Dimov, S.(1993) 'Separation of Isomeric Triacylglycerols by Silver Ion Thin-Layer Chromatography', Journal of Liquid Chromatography & Related Technologies, 16: 18, 3997 — 4008
To link to this Article: DOI: 10.1080/10826079308019682
URL: http://dx.doi.org/10.1080/10826079308019682

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 16(18), 3997-4008 (1993)

SEPARATION OF ISOMERIC TRIACYLGLYCEROLS BY SILVER ION THIN-LAYER CHROMATOGRAPHY

B. NIKOLOVA-DAMYANOVA, D. CHOBANOV, AND S. DIMOV
Laboratory of Lipid Chemistry
Institute of Organic Chemistry with Center of Phytochemistry
Bulgarian Academy of Sciences
1113 Sofia, Bulgaria

ABSTRACT

A procedure for the separation of positionally isomeric triacylglycerols by silver ion thin-layer chromatography at ambient temperature is described. The low silver ion content in the silica gel layer, the mobile phase of chloroform-methanol and the development in open cylindrical tanks are found essential for the improved resolution of the SMS-SSM, SMM-MSM, SDS-SSD species. Resolution of the DSD-DDS and DMD-DDM species is achieved also.

INTRODUCTION

Silver ion chromatography is of primary importance for the structural analysis of triacylglycerols (TG) [1-3]. TG are separated on the basis of a single property of their molecules - the nature of unsaturation. Most simple and most widely used is the resolution of a TG mixture according to the increasing number of double bonds [1-3].

Copyright © 1993 by Marcel Dekker, Inc.

Downloaded At: 08:11 25 January 2011

A few examples only have been reported where species containing isomeric fatty acids were distinguished (they were recently reviewed by one of us [3]) and of these only one deals with a real sample [4].

One of the most valuable achievement of silver ion chromatography is it's unique ability to resolve TG species which differ by the position of the fatty acid moieties in the glycerol backbone, i.e. species known as positionally isomeric TG [5-12]. Actually, these are TG of the type SSU-SUS and UUS-USU (S denote a saturated and U, unsaturated fatty acid). The so cold "symmetrical" TG, SUS, are typical for the most abundant plant fats and oils [1,14-16]. Resolutions of this type are, therefore, of great interest for the lipid analysis; the most important application being the characterization of confectionery fats [7, 10-13] and the detection of the adulteration of natural fats and oils with preesterified products [9]. Thin layer chromatography (TLC) was used in the first reports [5-7, 9] and only in the eighties' separations achieved by applying high performance liquid chromatography (HPLC) have been reported [8, 10-12].

The pairs SMS-SSM, SMM-MSM, SDS-SSD and SMD-MSD have been separated so far. Irrespective on the chromatographic technique used, the percentage of silver nitrate in the adsorbent was high, in the range 10% to 20% (w/w) [3]. Lower temperatures were recommended also [6, 8]. Generally, the resolutions achieved by using TLC were not clear enough [5, 9]. The best results obtained by HPLC were performed on a 3 micron silica column by applying a complex concave gradient of toluene/hexane and toluene/ethyl acetate mixtures [12].

The aim of this work was to show that resolutions of the same rank as these obtained by the modern HPLC methods can be performed by using simple TLC technique on ordinary silica gel layer, impregnated with methanolic silver nitrate solutions with concentrations which did not exceed 2%. Conditions have been found for the resolution of the pairs SDD-DSD and MDD-DMD. The quantities of the resolved isomeric TG can be measured by scanning densitometry.

Downloaded At: 08:11 25 January 2011

MATERIALS AND METHODS

All solvents used were reagent grade and were distilled before use. In addition, chloroform was treated to remove the stabilizing alcohol.

Silica gel, sulphuryl chloride and bromine were purchased from Merck.

Lard, sunflower oil and tristearin were commercial samples. They were randomized according to [17]. The TG fractions were isolated by preparative TLC (1.0 mm thick silica gel G layer and mobile phase petroleum ether acetone 100:8, v/v; detection by spraying with 2,7-dichlorofluorescein). The purified TG were dissolved in n-heptane to yield 0.3% solutions, which were stored at 4°C in the dark.

The silver ion TLC was performed exactly as described in [19]. In brief, 19x4 cm glass plates coated with ca. 0.2 mm layers of silica gel were impregnated with methanolic silver nitrate solution of specified concentration in the range 0.3% to 3.0%. Plates with 4% to 10% (w/w) silver nitrate were prepared by incorporation of the salt into the layer. 10 μl of the TG mixture were applied to the plate which was then developed by continuous ascending development with a mobile phase of specified composition and volume in open cylindrical jars (24 cm x 4.9 cm i.d.). After the development the plates were dried for 1 h at 110°C and treated consecutively with bromine and sulphuryl chloride vapours (30 min each). It was then heated on a metal plate with controlled temperature for 15 - 20 min at 200°C - 220°C. Finally, the plate was scanned by a Shimadzu CS-930 densitometer at 450 nm in zigzag reflection mode with a 1.2 mm x 1.2 mm slit.

RESULTS AND DISCUSSION

Randomized lard, sunflower oil and a mixture of sunflower oil and tristearin were selected as test samples. The fatty acid rearrangement during randomization leaded to the formation of all

Downloaded At: 08:11 25 January 2011

possible positionally isomeric TG in reasonable high quantities. The isomers were identified by cochromatographing the test samples with natural oils and fats with known composition of the isomeric TG. These test samples are particularly useful as standards for rapid identification of unknown TG mixtures [18].

The silica plates were home made. No special precautions were needed for their keeping. The layer was not activated before use. In our experience, preliminary activation has always been absolutely useless. Moreover, in case of activation, the separations became usually highly irreproducible.

The most contradictory point when using silver ion TLC in lipid analysis is the level of the silver nitrate content in the layer. In the most of the published procedures 5% to 20% are recommended [3]. The long practice of using silver ion TLC in our laboratory revealed, however, that a highly selective resolution can be achieved at much lower proportion of silver ions in the layer (the results have been recently reviewed in [3]). For the separation of positionally isomeric TG the problem was whether the 10% or 20% recommended in the literature [5-7,9] are of a real necessity. The both ways of introducing silver ions in the chromatographic system were tested: plates were either impregnated with 0.3% - 3.0% methanolic silver nitrate or the salt was incorporated into the silica gel layer in proportions from 4.0% to 10%, w/w.

Development proceeded in open cylindrical tanks of our own construction (see [19, 20] for details). We are convinced that these tanks are more effective then the conventional one. The fact that the tank remains open during the development enables the continuous migration of the solvent trough the layer to the upper edge of the plate where it evaporates. It becomes possible, therefore to vary the volume of the mobile phase and not only it's composition. Hence the volume of the mobile phase becomes a factor that affects the selectivity of the resolution. Relatively large volumes of mobile phases (8-20 ml) with low proportion of the polar component appear to be very selective and the separations achieved are better then those reported for silver ion TLC so far [3].

Downloaded At: 08:11 25 January 2011

Benzene, toluene, dichloroethane and chloroform as single solvents, and the respective mixtures with acetone, ethanol, methanol, ethyl acetate and buthyl acetate were tested as mobile phases. The single solvents did not produce reasonable separations and from the mixtures those based on chloroform or dichloroethane were found the best. From the oxygen-containing modifiers, buthyl acetate, ethyl acetate and acetone were not suitable. A secondary front was formed in presence of buthyl acetate; while mixtures with acetone were not selective enough and those with ethyl acetate provided badly shaped spots. The mixtures of chloroform with ethanol and methanol were found the best and these with methanol were slightly superior.

The results obtained so far, are illustrated on Figures 1-4, where densitograms of the separated isomers are presented. The respective experimental conditions are given in Table 1. Because of the specific features of silver ion TLC one could not expect to be able to resolve all isomeric TG under the same chromatographic conditions and on the same plate. Conditions were found, therefore, for each TG group separately, although the whole sample was always applied on the plate. A result of particular importance is the low concentration of the silver nitrate in the impregnating solution. It does not exceed 2% which were found necessary for the separation of DDS-DSD and DDM-DMD, while 0.5% were sufficient for the separation of SSM and SMS. Two successive developments and two mobile phases, the second with lower methanol proportion and larger volume, were needed to resolve DDS from DSD and DDM from DMD (Fig. 4). As far as we know separation of these isomers has not been reported as yet. No conditions were found under which MMD-MDM and SMD-MSD-SDM could be resolved.

The resolutions presented here are better then the reported previously for silver ion TLC and are comparable with some of those obtained by silver ion HPLC [8,11].

Apparently, the high silver nitrate content in the sorbent commonly used in silver ion TLC and HPLC is definitely not essential for the resolution of positionally isomeric TG. Low temperatures are also not necessary. The use of a more sophisticated chromatographic

Downloaded At: 08:11 25 January 2011

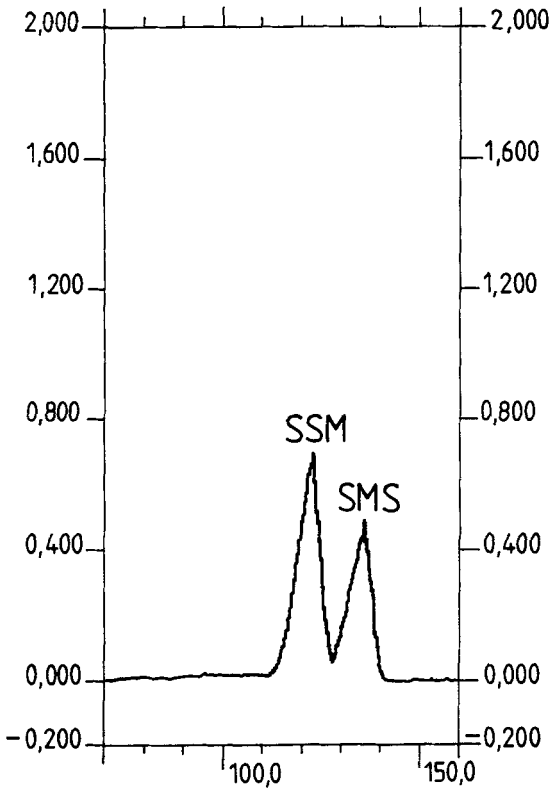


FIGURE 1. Resolution of SSM and SMS TG species. For the experimental conditions see Table 1.

technique, as HPLC, imroves the resolution in some cases and shortens the time of analysis but the results are generally similar.

We assume that a factor which affected the resolutions achieved in this work was the development of the plates. It was performed in open cylindrical tanks. The solvent gradient that is assumed to exist in silica gel TLC [21] should play a significant role since in the open tank no equilibration and saturation between the solvent vapours and the layer can occur. Presumably, under such conditions the composition of the mobile phase alongside the height of the plate differs

Downloaded At: 08:11 25 January 2011

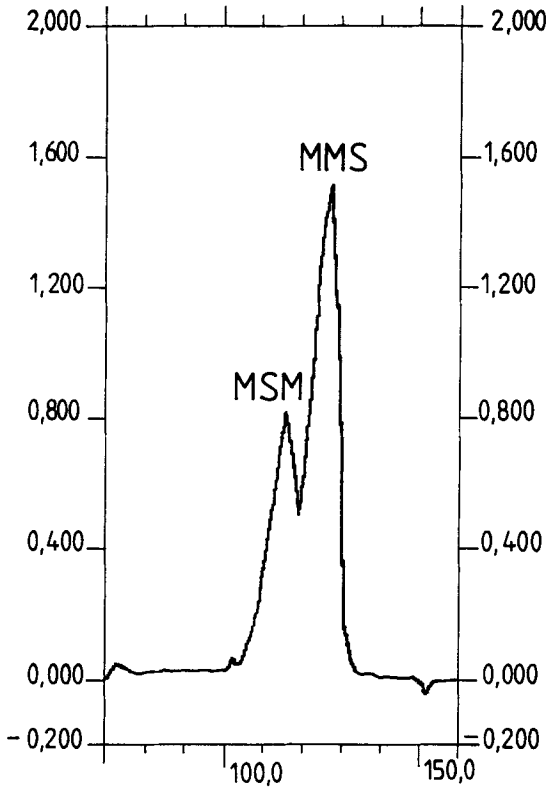


FIGURE 2. Resolution of MMS and MSM TG species. For the experimental conditions see Table 1.

considerably with the proportion of the more polar component becoming very low or even zero with increasing height. The low silver ion content in the layer and the low polarity of the mobile phase act in the same direction, i.e. they help to materialize the fine difference between the positions of the fatty acids in the glycerol backbone.

As discussed elsewhere [3], this "open" system is sensitive toward the laboratory environment but can be kept under control in order to provide reproducible separations.

Downloaded At: 08:11 25 January 2011

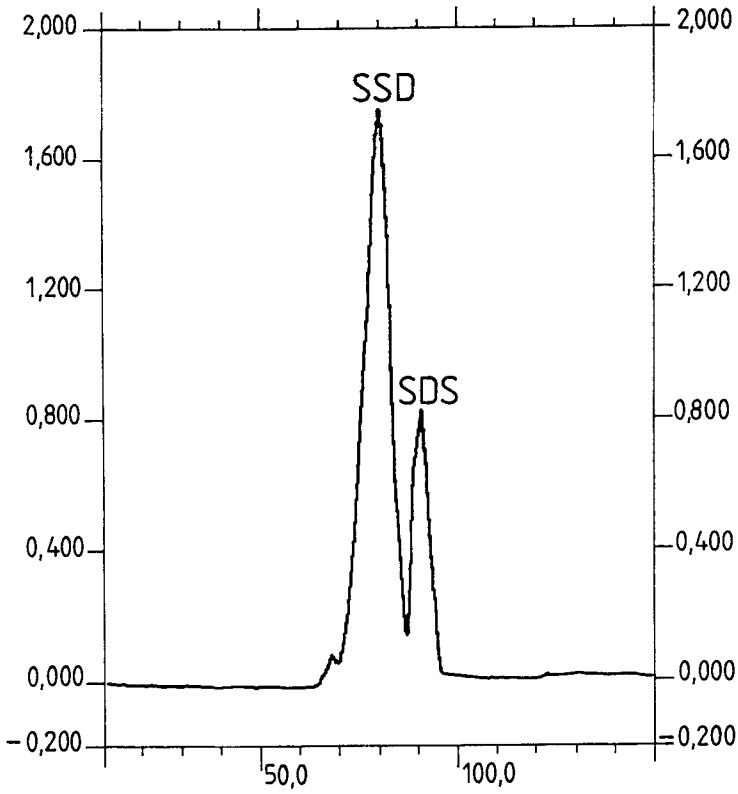


FIGURE 3. Resolution of SSD and SDS TG species. For the chromatographic conditions see Table 1.

The migration order of the isomeric species established in this work agrees well with those reported by others [5-12]. The fact that it does not depend on the chromatographic technique used reveal that it is governed by a basic property of the TG molecule. It has been stated [1], for example, that the symmetric species migrate always ahead the unsymmetric. This is true for SMS, SDS, DSD and DMD species but not for MMS which migrate ahead of MSM (see also [9]). It has been suggested [9], therefore, that the migration order depends on the type of the fatty acids which occupy the 1,3-position in glycerol. Thus, in

Downloaded At: 08:11 25 January 2011

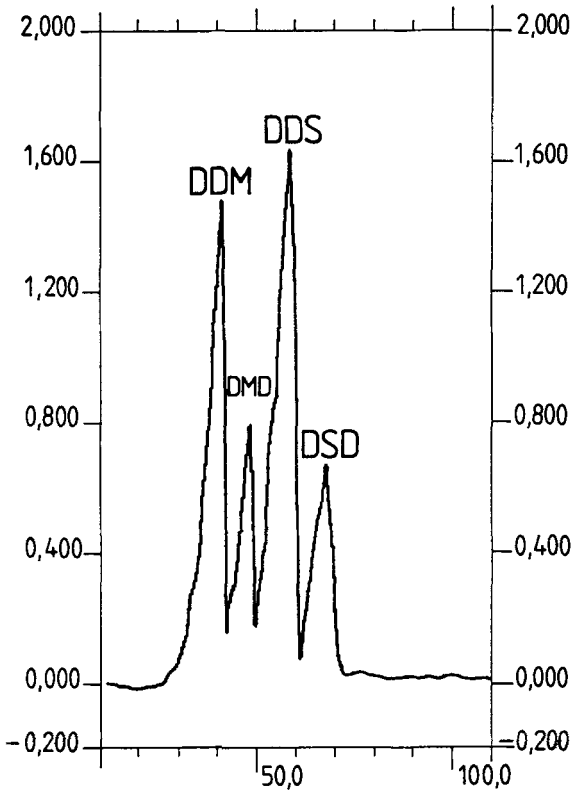


FIGURE 4. Resolution of DSD, DDM, DMD and DDM TG species. For the chromatographic conditions see Table 1.

case an unsaturated fatty acid occupies one of these positions the molecule forms stronger π-complex with silver ions and is held stronger by the support. The migration of the DSD and DMD species found in this work does not agree with this suggestion as they migrate ahead and not behind the DDS and DDM species, respectively. It seems, therefore, that a factor of more complex origin governs the separation. It is presumably, based on the steric or/and electronic state of the TG molecule and depends on the type of the fatty acids.

Downloaded At: 08:11 25 January 2011

TABLE 1.
Separation of Positionally Isomeric Triacylglycerols by Silver Ion Thin-Layer Chromatography.

Isomers[a]	Reference mixture[b]	Migration distance, mm	Chromatographic Conditions		
			Silver nitrate[c], %	Methanol, %[d]	Volume, ml[e]
SSM, SMS	RL	120, 135	1	0.5	12
MMS, MSM	RL	118, 113	1	1.0	14
SSD, SDS	R(Sf+S3)	87, 76	1	1	16
DDS, DSD[f]	RSf	57 49	2	I -2.5	6
				II - 1.5	15
DDM, DMD	RSf	40 33	2	I - 2.5	6
				II - 1.5	15

[a] S - saturated, M - monoenoic and D -dienoic fatty acid.
[b] RL - randomized lard, RSf - randomized sunflower oil, R(Sf+S) - randomized mixture of sunflower oil and tristearin.
[c] Concentration of the methanolic silver nitrate used to impregnate the plates.
[d] Proportion of methanol in the chloroform methanol mixture used as a mobile phase.
[e] Volume of the mobile phase.
[f] Plates were developed twice, I, denotes the first and II - the second development.

Finally, because of the low silver nitrate content in the layer, the background of the plate remained white after the TG spots were charred and scanning densitometry could be successfully applied. The charring procedure that includes successive treatment of the plate with bromine and sulphuryl chloride vapours ensures quantitative results that are both reproducible and accurate as was already shown [22].

We assume that the separations discussed here present an easy to perform and inexpensive alternative to the more sophisticated HPLC methods. The results are unambiguous and can be always confirmed by cochromatography with suitable standards.

ACKNOWLEDGEMENT

This project was supported in part by the National Foundation for Scientific Research under Contract No X-15.

Downloaded At: 08:11 25 January 2011

REFERENCES

1. C. Litchfield, Analysis of Triglycerides, Academic Press, New York, 1972.

2. W. W. Christie, Lipid Analysis, Pergamon Press, Oxford, 1982.

3. B. Nikolova-Damyanova, Silver Ion chromatography and Lipids, in Advances in Lipid Methodology, W. W. Christie, ed., The Oily Press, Ayr, 1992, pp. 181 - 327.

4. B. Nikolova-Damyanova. W. W. Christie, B. Herslof, J. Am. Oil Chem. Soc., 67:503-507 (1990).

5. C. B. Barret, M.S.J. Dallas, F. B. Padley, J. Am. Oil Chem. Soc., 40: 580-584 (1963).

6. H. Wessels, N.S.Rajagopal, Fette Seifen Anstrichmittel, 71: 543-552 (1969)

7. M. S. J. Dallas, F. B. Padley, Lebensm.-Wiss. Technol. 10: 328-331 (1977).

8. E. C. Smith, A. D. Jones, E. W. Hammond, J. Chromatogr., 188: 209-212 (1980).

9. D. Gegiou, M. Georgouli, J. Am. Oil Chem. Soc., 60: 833-835 (1983).

10. O. Podlaha, B. Toregard, Fette Seifen Anstrichmittel, 86: 243-245 (1984).

11. S. Takano, Y. Kondoh, J. Am Oil Chem. Soc,, 64: 380-383 (1987).

12. B. S. J. Jeffrey, J. Am Oil Chem. Soc., 68: 289-293 (1991)

13. B. Nikolova-Damyanova, B. Amidzhin. Bulg. Chem. Commun., in the press.

Downloaded At: 08:11 25 January 2011

14. A. J. Sheppard, J. L. Iverson, J. L. Weihrauch, in Handbook of Lipid Research - 1. Fatty Acids and Glycerides, A.Kuksis ed., Plenum Press, New York, 1978, pp341-347.

15. H. P. Kaufmann, H. Wessels, Fette seifen Anstrichmittel, 66: 81-86 (1964).

16. H. P. Kaufmann. H. Wessels, Fette Sefen Anstrichmittel, 68: 249-255 (1966).

17. D. Chobanov, M. Topalova, J. Am. Oil Chem. Soc., 56: 581- (1979)

18. B. Nikolova, N. Hien, D. Chobanov, S. Dimov, in Proceedings F.E.C.S. 3rd Int Conference Chemistry & Biotechnology of Biologically Active Natural Products, 5: 1232-1234 (1987), publ. Veincheim, Germany.

19. D. Chobanov, R. Tarandjiska, D. Chobanova, J. Am. Oil Chem. Soc., 53: 48-51 (1976).

20. D. Chobanov, R. Tarandjiska, B. Nikolova-Damyanova, J. Planar Chromatogr.-Mod. TLC, 5: 157-163 (1992).

21. D. Nurok, Chem Rev., 89: 363-375 (1989).

22. B. Nikolova-Damyanova. B. Amidzhin, J. Planar Chromatogr.-Modern TLC, 4: 397-401 (1991).

Received: April 2, 1993
Accepted: April 15, 1993

Downloaded At: 08:11 25 January 2011