



Comparison of iso-elutotropic mobile phases at different temperatures for the separation of triacylglycerols in Non-Aqueous Reversed Phase Liquid Chromatography



Dorra Hmida^{a,b,c}, Manef Abderrabba^b, Alain Tchaplal^a, Sylvie Héron^{a,*}, Fathi Moussa^a

^a Univ Paris-Sud, Lip(Sys)², LETIAM^A, IUT d'Orsay, Plateau de Moulon, 91400 Orsay, France

^b Institut Préparatoire aux Etudes Scientifiques et Techniques (IPEST), Laboratoire Matériaux, Molécules et Applications (LMMA), BP 51 La Marsa 2070, Tunisia

^c Université de Tunis El Manar, Faculté des Sciences de Tunis, Campus Universitaire 2092, El Manar, Tunis, Tunisia

ARTICLE INFO

Article history:

Received 3 February 2015

Accepted 11 March 2015

Available online 19 March 2015

Keywords:

Non-aqueous reversed phase

Triacylglycerols

Eluotropic solvent strength scale

Iso-eluotropic mobile phases

Butanol

Temperature effect

ABSTRACT

Triacylglycerols (TAGs) are a large class of neutral lipids that naturally occur in both plant and animal oils and fats. Their analyses in Non-Aqueous Reversed Phase Liquid Chromatography (NARP) require a mixture of weak solvent (mostly acetonitrile) and strong solvent. In the present work, we have established eluotropic solvent strength scale of several binary mobile phases on C₁₈ bonded silica at different temperatures (acetonitrile/methylene chloride, acetonitrile/acetone, acetonitrile/ethyl acetate, acetonitrile/propan-2-ol, and acetonitrile/butan-1-ol at 25 °C, 43 °C, 63 °C and 85 °C); it is based on the methylene selectivity and the use of homologous series. We show that this scale is well suited to the TAGs analysis. The analysis of nine seed oils (*Aleurites fordii*, *Calophyllum inophyllum*, *Glycina max*, *Olea europea*, *Orbignya olifera*, *Pinus koraiensis*, *Pistacia lentiscus*, *Punica granatum* and *Ribes nigrum*) in iso-eluotropic conditions leads to propose unambiguously the couple MeCN/BuOH at 25 °C as the best system to separate TAGs. The use of butanol, as strong solvent, provides very good TAGs congeners separations and avoids the use of chlorinated solvents which gave to this day the best separations.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Triacylglycerols (TAGs) consist of the esterification of one glycerol backbone with three fatty acid residues. They are a large class of neutral lipids that naturally occur in both plant and animal oils and fats. The complex composition of lipids, the great number of congeners to be separated and their widespread occurrence make them extremely challenging to analyze.

Several strategies are applied to the investigation of TAG species. Among them the non-aqueous reversed-phase HPLC (NARP-LC) is commonly used. The elution order is mainly based on partition number (PN) which is equivalent to the total carbon number (CN) minus two times of the double bond number (DB), (i.e. $PN = CN - 2DB$) [1]. Using the efficient reversed stationary phases currently available and the optimized mobile phase compositions, TAGs with the same PN (critical pair) can usually be separated [2]. Various grafted RP stationary phases such as particles of C₁₈ bonded

porous silica are mainly employed [3]. C₂₂ [4] and C₃₀ grafts instead of C₁₈ grafts as well as C₁₈ bonded monolithic porous silica have also been tested [5]. Mobile phases are composed of a weak organic solvent (as methanol, acetonitrile, propionitrile and nitromethane), mostly acetonitrile (MeCN) and a strong organic one, mostly chlorinated solvents, acetone, THF or propanol 2 [3,5–10]. Ternary mobile phases (combining two strong solvents with MeCN) are also proposed specially for animal fats [11] but also reviewed since a long time for vegetable oils [12]. Studies on the role of temperature [5] with different alcohols as strong solvents [13] have also been reported. At last, the high efficiency of the non-porous core columns has also been investigated [13–16]. In previous works [3–16], the authors propose or report the best chromatographic system for their particular investigated oils and fats, consequently, it is difficult to compare these results to find the best single chromatographic system, i.e. stationary phase, mobile phase composition, and temperature for global separation of a large as possible range of TAGs with different molecular structures.

In this work, we propose a comparison of several binary mobile phases associated with different temperatures leading to separations of triacylglycerols of various oils. The comparison will be done in iso-eluotropic conditions.

* Corresponding author. Tel.: +33 1 69 33 61 33.

E-mail address: sylvie.heron@u-psud.fr (S. Héron).

^A Formerly Groupe de Chimie Analytique de Paris-Sud EA 4041.

Table 1
Nomenclature.

Abbreviation	Name	Carbon number: number of double bonds
Co	Caproyl	6:0
Cy	Caprylyl	8:0
C	Capryl	10:0
La	Lauryl	12:0
M	Myristyl	14:0
P	Palmityl	16:0
Po	Palmitoleyl	16:1 (Z9)
S	Stearyl	18:0
O	Olelyl	18:1 (Z9)
L	Linoleyl	18:2 (Z9, Z12)
T	Taxoleyl	18:2 (Z5, Z9)
Ln	Linolenyl	18:3 (Z9, Z12, Z15)
γ Ln	γ Linolenyl	18:3 (Z6, Z9, Z12)
α E	α eleostearyl	18:3 (Z9, E11, E13)
β E	β eleostearyl	18:3 (E9, E11, E13)
Pu	Punicyl	18:3 (Z9, E11, Z13)
Pi	Pinolenyl	18:3 (Z5, Z9, Z12)
St	Stearidonyl	18:4 (Z6, Z9, Z12, Z15)
Go	Gondoleyl	20:1 (Z11)
Sc	Sciadopinoleyl	20:3 (Z5, Z11, Z14)

Triacylglycerols are named according to the following fatty acid abbreviations.

The paper is divided into two parts. In the first one, we establish the eluotropic strength scale of these different binary mobile phases at different temperatures. In the second part, we use this scale to work in iso-eluotropic conditions and to compare TAGs separations of oils of different compositions, in these different chromatographic systems.

2. Materials and methods

2.1. Reagents and samples

All solvents were HPLC grade. Acetonitrile (MeCN) was purchased from Baker (Deventer, The Netherlands), methylene chloride (CH_2Cl_2) from Carlo Erba (Rodano, Italy), acetone (AcMe), ethyl acetate (AcOEt), propan-2-ol (iPrOH) and butan-1-ol (BuOH) from Merck (Darmstadt, Germany).

Pure standards compounds, tricaproin (CoCoCo), tricaprylin (CyCyCy), tricaprinn (CCC), trilaurin (LaLaLa), trimyristin (MMM), tripalmitin (PPP), tristearin (SSS), triolein (OOO), trilinolein (LLL), trilinolenin (LnLnLn) were from Cluzeau (Sainte Foy la Grande, France) or Larodan (Malmö, Sweden). Abbreviations are described Table 1.

Calophyllum inophyllum oil (tamanu oil) and *Ribes nigrum* oil (black currant seed oil) were from Pierre Fabre research laboratory, *Glycina max* oil (soya oil) was bought in supermarket, *Orbignya olifera* (babassu oil) and *Punica granatum* (pomegranate oil) oils from Aroma-zone (Paris, France) and *Aleurites fordii* oil (tung oil) from Laverdure (Paris, France). *Olea europea* (olive oil) and *Pistacia lentiscus* (pistachio oil) were from Tunisia. *Pinus koraiensis* seed oil (pine nut oil) was kindly given by R.L. Wolff (ISTAB, Univ. Bordeaux I, Talence, France).

All samples were dissolved in MeCN/ CH_2Cl_2 (50/50 v/v) and have been injected at least three times.

2.2. UHPLC instrument and working conditions

The UHPLC system consisted of a binary pump, an automated injector and a column oven, (Agilent 1200 Series, Massy, France) coupled to a model Sedex 85 evaporative light-scattering detector (Sedere, Alfortville, France). The nebulizing gas was air at 3.0 bars, the nebulization temperature was 40 °C and the gain PM was 11. Chromatograms were recorded with Chemstation acquisition software (Agilent Technologies, Massy, France).

Table 2
Iso-elutotropic conditions corresponding to $\epsilon^0 = 3$.

Mobile phase	T (°C)
MeCN/BuOH 74/26	25
MeCN/AcMe 50/50	25
MeCN/AcOEt 66/34	25
MeCN/iPrOH 66/34	25
MeCN/BuOH 83/17	43
MeCN/AcMe 69/31	43
MeCN/AcOEt 79/21	43
MeCN/iPrOH 77/23	43
MeCN/BuOH 96/4	63
MeCN/AcMe 92/8	63
MeCN/AcOEt 95/5	63
MeCN/iPrOH 94/6	63

2.2.1. Determination of the eluotropic strength

An Uptisphere Strategy 100 Å C18-2 column (50 × 2.1 mm, 1.7 μm) was used (Interchim, Montluçon, France). The flow rate was 0.2 mL/min.

The different mobile phases were: MeCN/ CH_2Cl_2 from 100/0 to 50/50 (v/v) at 25 °C; MeCN/AcMe, MeCN/AcOEt, MeCN/iPrOH and MeCN/BuOH from 100/0 to minimum 70/30 or 20/80 maximum (v/v) at 25 °C, 43 °C, 63 °C and 85 °C.

For higher volume percentages of strong solvent, the used homologous TAGs for establishing the nomogram of eluent strength have very weak retention and accuracy of deduced eluent strength becomes weak. For overcoming that, the homologous triacylglycerols with higher carbon number must be chosen as probes. But it was previously demonstrated that the corresponding values of the methylene selectivity is lower for such group of homologues which lead to a slight change of eluent strength for higher homologues comparing to smaller ones [17]. So we have decided to keep the same standards for all the conditions and not to determinate eluent strength higher than 3.1. Moreover, the range of tested solvent eluotropic strength (2.75–3.1) allows analyzing most of vegetable oils and fats.

2.2.2. Iso-elutotropic conditions

An Uptisphere Strategy 100 Å C18-2 column (100 × 2.1 mm, 1.7 μm) was used (Interchim, Montluçon, France). The flow rate was 0.25 mL/min for olive, pistachio, soya, and tamanu oils or 0.15 mL/min for babassu, black currant seed, pine nut, pomegranate and tung oils.

The iso-elutotropic conditions corresponding to $\epsilon^0 = 3$ are given Table 2.

For each TAG, retention factor has been calculated: $k = (t_r - t_0)/t_0$, with t_r retention time of the TAG and t_0 the column dead time determined by the weighing method [18].

2.3. Identification of TAGs

TAGs have been identified as described in the article of Acheampong et al. [7]. This methodology uses both mass spectrometry and different retention laws available for TAGs. For oils with TAGs with polyunsaturated fatty acids, mainly C18:3 with the three double bonds differently located inside the fatty alkyl chain, mass spectrometry is inefficient for their characterization, so we have used the method recently published using TAGs retention laws on unsaturated TAGs isomers [19].

2.4. Criteria leading to the selection of the optimal mobile phase.

The used methodology will be discussed later in the text. Different used criteria to evaluate the quality of the chromatograms are [20]:

Download English Version:

<https://daneshyari.com/en/article/1212218>

Download Persian Version:

<https://daneshyari.com/article/1212218>

[Daneshyari.com](https://daneshyari.com)