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ORIGINAL PAPER

Comparative Evaluation of Vegetable Oils-Impregnated Layers as Reversed-Phases for Thin-layer Chromatography

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Abstract Silica gel plates impregnated with a variety of oils including vegetable oils (olive, sunflower and corn oil) and synthetic oils (trioctylamine and paraffin oil) were evaluated and compared with the commercially available reversed-phases TLC plates (RP-18, RP-18W, and CN). A series of selected lipophilic vitamins was employed to evaluate the suitability of oils as reversed-phases for TLC and to provide different lipophilicity indices: R_{M0}, scores corresponding to the first principal component of R_F and/or R_M, the arithmetic mean of R_F and R_M values obtained with solvent mixture containing various concentrations of methanol in water. The retention results were excellent ($r > 0.98$) and allowed accurate estimation of lipophilicity of selected vitamins and to ranking the lipophilicity of oils when comparing with chemically bonded phases. Concerning the lipophilicity scale of vegetable oils, it is worth noting that corn oil presents the highest lipophilicity, closely followed by the olive and sunflower oils.

Keywords Lipophilicity · Vegetable oils · Lipophilic vitamins · RP-HPTLC · PCA

Introduction

Lipophilicity refers to the ability of a chemical compound to dissolve in fats, oils, lipids, and non-polar solvents such as hexane or toluene. Thus, lipophilic substances tend to dissolve in other lipophilic substances, while hydrophilic substances tend to dissolve in water and other hydrophilic substances. According to the IUPAC, lipophilicity represents the affinity of a molecule or a moiety for a lipophilic environment [1]. It is commonly measured by its distribution behavior in a biphasic system, either liquid–liquid (e.g., partition coefficient in *n*-octanol/water K_{ow}) or solid/liquid (retention on reversed-phase high performance liquid chromatography or thin-layer chromatography system).

This particular property plays an important role in several ADME (absorption, distribution, metabolism and elimination) aspects, as well as in the pharmacodynamic and toxicological profile of drugs; it is the single most informative and successful physicochemical property in medicinal chemistry [2]. The success of the partition coefficient (log K_{ow}) in quantitative structure–activity relationships (QSAR), quantitative structure–property relationships (QSPR) and quantitative structure-retention relationships (QSRR) is well established [3–5].

Determination of the partition coefficient by the equilibration method using classical shake-flask technique has a series of disadvantages (is very tedious, requires relatively large amounts of pure solutes to be examined, and it is limited to log K_{ow} values between -2 and $+4$) and has been successfully replaced by chromatographic methods. The advantages of reversed-phase high performance thin layer chromatography (RP-HPTLC) methods consist of the very small amounts of samples needed for the estimation and the less strict requirement of purity because the impurities separate during the chromatographic process. They are rapid and relatively simple, low in cost, and easy to perform. In addition, we have to stress the dynamic aspect of the chromatographic process and the wide choice of stationary phases and developing solvents. A lot of lipophilicity studies were based on RP-18 stationary phases

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and good correlation between log K_{ow} and R_{M0} or isocratic R_M values were related [6, 7]. Furthermore, the possibility of impregnation of the HPTLC plates with a series of natural or synthetic materials including oils (more or less similar with the lipidic biological membranes) might be one of the most realistic alternatives. The oils most employed for modification of silica gel plates were paraffin oil [8–12], silicon oil [13–16] and ethyl oleate [17]. The chemical composition of the vegetable oils indicates a large amount of triglycerides, free fatty acids (especially oleic and linoleic acid), phytosterols, lipophilic vitamins and traces of minerals [18].

The purpose of this work was to investigate the feasibility of silica gel plates impregnated with a variety of vegetable and synthetic oils and to compare them with the commercially available reversed-phases HPTLC plates. In this order, the lipophilicity of some lipophilic vitamins was determined using mobile phases containing various concentrations of methanol in water and estimated by different indices. In addition, the scores obtained applying principal component analysis (PCA) offer the possibility to get a new lipophilicity scale and the lipophilicity chart of compounds and reversed-phases investigated; eigenvalues and eigenvectors (loadings) giving new insights into the chromatographic mechanism and the behavior of compounds.

Theory

RP-HPTLC provides a variety of indices (descriptors) that can be used as lipophilicity estimators. The most popular lipophilicity indices measured by RP-HPTLC are derived by the retention R_F values according to linear relationship described by the Soczewiński-Wachtmeister equation [6]:

$$R_M = R_{M0} + bC, \quad (1)$$

where R_M is defined by Bate-Smith and Westall [19] through the following formula

$$R_M = \log\left(\frac{1}{R_F} - 1\right). \quad (2)$$

The R_{M0} indicates the extrapolated value of the pure water as the mobile phase and it is the HPTLC descriptor most frequently used in QSAR/QSPR/QSRR analysis; b represents the slope and it is directly related to the specific surface area of the stationary phase, while C represents the volume fraction of the organic solvent in the mobile phase. The slope of the linear regression equation is also considered as an alternative descriptor of lipophilicity. The scale of lipophilicity, based on the isocratic retention factors, has been preferred by some authors since it requires fewer experiments. However, linear extrapolation is generally used to obtain R_{M0} values as more representative lipophilicity indices, their values being of the same order of magnitude as n-octanol–water log K_{ow}. The arithmetic mean of R_M (mR_M) and/or R_F (mR_F) values obtained for all values of C may be used as well as lipophilicity indices. It is also possible to obtain a new lipophilicity scale by applying PCA directly to the matrix of retention data for all compounds and combinations of methanol–water (R_F and/or R_M values obtained for all values of C). The aims of PCA are to extract meaningful and interpretable features from the underlying information of the multivariate raw data. PCA has the ability to separate the relevant information from the noise. Usually, the first few components account for all information in raw data. The characteristics of each principal component are the scores (in our case, linear combinations of retention indices) relating to the investigated compounds and loadings (contribution of the raw variable or measurement to each component).

Taking into account the major breakdowns of regression approach (extrapolation, linearity or non-linearity of R_M against the volume of fraction of the polar solvent at small concentration of the polar solvent), the scores corresponding to the first principal component (PC1) and/or arithmetic means mentioned above appeared to be one of the illuminating solutions for the lipophilicity scale resulting from retention data. In addition, a careful investigation of eigenvalues and eigenvectors (loadings) can offer useful information concerning the chromatographic behavior of the compounds and their retention mechanism [20–24].

Materials and Methods

Materials

The β-carotene, lycopene, lutein, astaxanthin, 9-*cis*-retinal, all-*trans*-retinal and δ-tocopherol standards were obtained from Sigma (Redox, Bucuresti), while zeaxanthin, retinol and retinoic acid were Fluka (Redox, Bucuresti) products; α and γ-tocopherols were purchased from Acros Organics (Redox, Bucuresti). The solvents used (chloroform, methanol, diethyl ether) were obtained from Chimopar (Bucuresti). All the chemicals were of analytical grade. The trioctylamine (TOA) and all the HPTLC plates (10 × 10 cm, F_{254}) were purchased from Merck (Nordic Invest, Cluj-Napoca). The spotting was performed using a Hamilton microsyringe of 10 μL. The paraffin, olive, sunflower and corn oil were from the local market.

Chromatography

The standard solutions were prepared in chloroform (1 mg mL^{-1}). The spots (1 μL) were applied 1.5 cm from

Table 1 The lipophilicity indices obtained on RP-18, RP-18W, CN and oil impregnated plates

No	Compound	RP-18					RP-18W					CN					Paraffin				
		mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M
1	β-Carotene	0.035	1.489	16.895	0.443	−1.944	0.149	0.815	9.883	0.451	−1.134	0.071	1.170	5.462	0.685	−1.973	0.115	0.930	19.653	0.811	−1.945
2	Lycopene	0.035	1.498	18.450	0.443	−1.972	0.212	0.632	10.680	0.323	−0.750	0.159	0.784	5.942	0.489	−1.136	0.144	0.791	12.207	0.753	−1.656
3	Lutein	0.170	0.690	4.974	0.141	−0.121	0.314	0.360	7.633	0.094	−0.115	0.277	0.478	6.693	0.229	−0.479	0.823	−0.669	4.015	−0.606	1.269
4	Astaxanthin	0.252	0.475	4.434	−0.041	0.357	0.366	0.250	6.530	−0.024	0.139	0.288	0.425	5.006	0.203	−0.333	0.797	−0.596	5.125	−0.553	1.121
5	Zeaxanthin	0.153	0.750	6.283	0.181	−0.259	0.301	0.390	8.145	0.122	−0.189	0.277	0.473	6.484	0.229	−0.462	0.832	−0.699	5.508	−0.624	1.325
6	Retinol	0.551	−0.089	4.143	−0.711	1.605	0.589	−0.158	2.790	−0.531	1.079	0.633	−0.240	1.769	−0.570	1.187	0.867	−0.837	12.486	−0.693	1.591
7	Retinoic Acid	0.399	0.178	2.423	−0.370	1.021	0.528	−0.049	4.255	−0.390	0.822	0.547	−0.084	2.242	−0.378	0.836	0.823	−0.672	6.410	−0.604	1.270
8	9-*cis*-Retinal	0.323	0.322	3.595	−0.201	0.699	0.508	−0.015	4.256	−0.348	0.748	0.463	0.065	2.653	−0.190	0.501	0.493	0.012	7.633	0.055	−0.095
9	All-*trans* Retinal	0.317	0.334	3.077	−0.187	0.673	0.509	−0.015	4.321	−0.347	0.747	0.478	0.040	2.452	−0.222	0.562	0.503	−0.006	7.824	0.035	−0.060
10	α-Tocopherol	0.150	0.757	5.900	0.186	−0.273	0.232	0.566	9.560	0.278	−0.590	0.447	0.098	3.360	−0.152	0.415	0.185	0.662	13.400	0.672	−1.401
11	γ-Tocopherol	0.175	0.676	4.950	0.130	−0.090	0.266	0.484	9.625	0.203	−0.414	0.451	0.089	2.878	−0.162	0.443	0.287	0.410	14.318	0.471	−0.900
12	δ-Tocopherol	0.240	0.501	3.633	−0.016	0.303	0.280	0.451	9.696	0.171	−0.345	0.451	0.089	3.178	−0.162	0.439	0.383	0.218	16.194	0.282	−0.519
		TOA					Olive					Sunflower					Corn				
		mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M
1	β-Carotene	0.016	1.827	10.547	0.753	−2.812	0.058	1.296	11.001	0.719	−2.197	0.062	1.203	6.460	0.748	−2.057	0.065	1.322	15.882	0.662	−2.171
2	Lycopene	0.020	1.731	9.341	0.747	−2.618	0.067	1.223	10.808	0.699	−2.037	0.074	1.125	7.083	0.720	−1.889	0.058	1.328	13.298	0.678	−2.156
3	Lutein	0.600	−0.180	4.321	−0.417	1.206	0.506	−0.009	5.338	−0.286	0.733	0.566	−0.118	3.917	−0.379	0.892	0.496	0.008	5.224	−0.303	0.835
4	Astaxanthin	0.798	−0.601	3.113	−0.810	2.051	0.439	0.108	3.172	−0.134	0.493	0.455	0.081	3.914	−0.130	0.450	0.417	0.149	4.600	−0.126	0.531
5	Zeaxanthin	0.593	−0.165	3.616	−0.402	1.178	0.494	0.011	4.695	−0.259	0.696	0.532	−0.058	5.246	−0.303	0.751	0.493	0.014	4.937	−0.295	0.825
6	Retinol	0.613	−0.204	4.897	−0.444	1.253	0.793	−0.598	4.069	−0.927	2.048	0.790	−0.584	3.390	−0.879	1.931	0.771	−0.539	4.214	−0.917	2.048
7	Retinoic acid	0.758	−0.502	3.735	−0.732	1.851	0.723	−0.431	4.945	−0.770	1.675	0.766	−0.522	2.847	−0.826	1.796	0.722	−0.420	2.812	−0.808	1.800
8	9-*cis*-Retinal	0.522	−0.039	5.352	−0.261	0.922	0.428	0.126	2.665	−0.110	0.458	0.447	0.095	3.394	−0.112	0.423	0.391	0.200	5.850	−0.069	0.404
9	All-*trans* Retinal	0.520	−0.036	5.326	−0.259	0.917	0.432	0.120	2.570	−0.117	0.472	0.459	0.072	3.546	−0.140	0.472	0.396	0.192	5.676	−0.079	0.425
10	α-Tocopherol	0.074	1.165	12.560	0.635	−1.497	0.140	0.877	11.896	0.534	−1.279	0.139	0.887	12.369	0.577	−1.396	0.120	0.928	9.862	0.539	−1.241
11	γ-Tocopherol	0.085	1.080	10.411	0.614	−1.322	0.191	0.671	8.695	0.420	−0.798	0.179	0.743	11.948	0.485	−1.073	0.165	0.765	10.171	0.438	−0.885
12	δ-Tocopherol	0.103	0.984	9.865	0.577	−1.130	0.276	0.438	6.580	0.229	−0.264	0.290	0.410	7.159	0.239	−0.300	0.235	0.556	9.214	0.281	−0.413

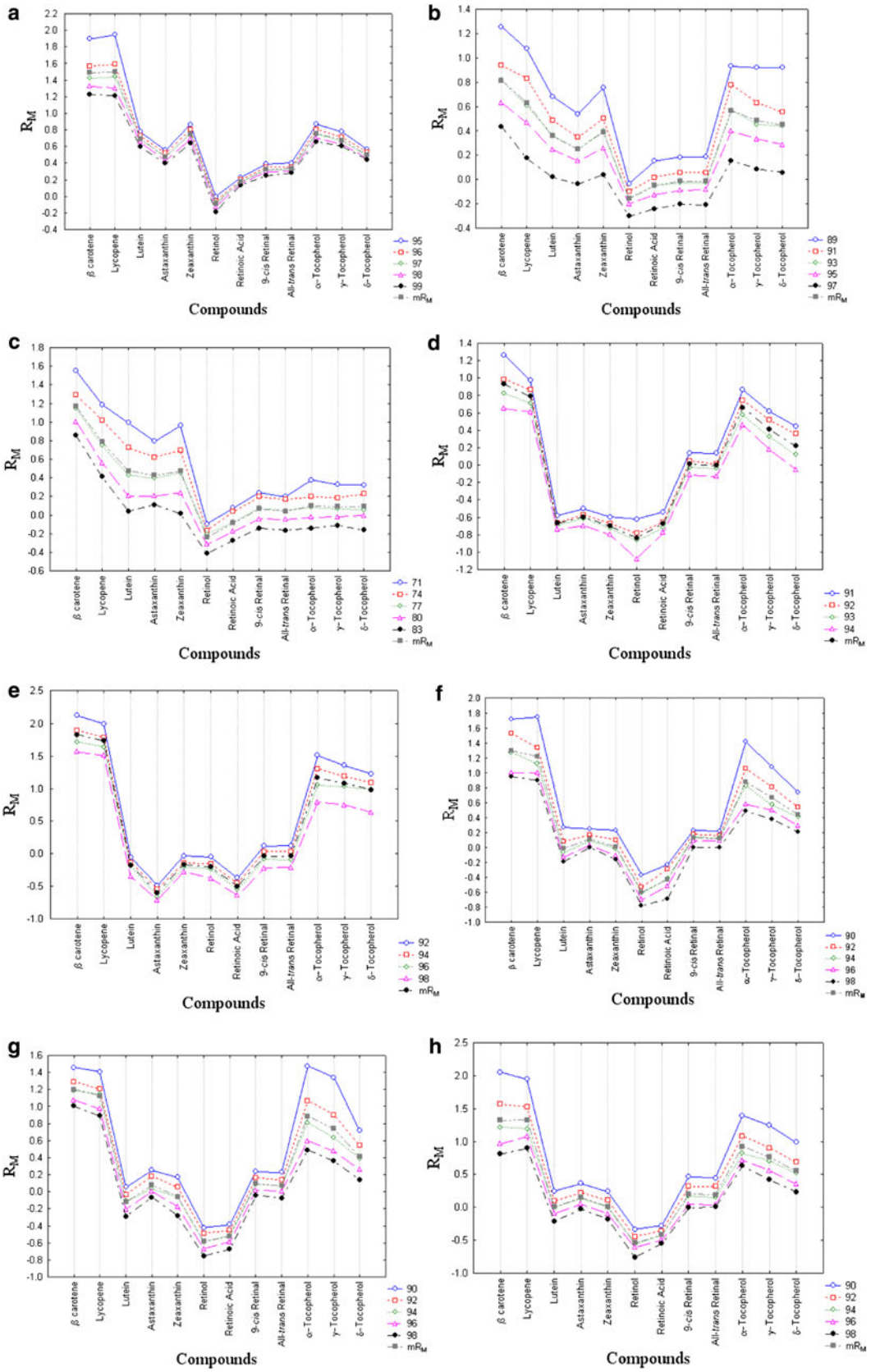

Fig. 1 Profiles of R_M for all fractions of methanol on the investigated stationary phases: RP-18 (**a**); RP-18W (**b**); CN (**c**); paraffin (**d**); TOA (**e**); olive (**f**); sunflower (**g**); corn (**h**)

Table 2 Correlation matrix of the lipophilicity indices obtained on TOA, olive, sunflower and corn oil-impregnated plates vs. RP−18, RP−18W, CN and paraffin oil impregnated plates

	TOA					Olive					Sunflower					Corn				
	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M
mR_F/RP-18	0.65	−0.70	−0.54	−0.65	0.70	0.86	−0.86	−0.70	−0.86	0.86	0.83	−0.84	−0.53	−0.83	0.84	0.83	−0.85	−0.76	−0.83	0.84
mR_M/RP-18	−0.69	0.79	0.55	0.69	−0.79	−0.84	0.89	0.76	0.84	−0.89	−0.81	0.86	0.42	0.81	−0.85	−0.80	0.87	0.86	0.80	−0.88
R_{M0}/RP-18	−0.61	0.75	0.46	0.61	−0.75	−0.67	0.78	0.69	0.67	−0.78	−0.66	0.74	0.23	0.67	−0.73	−0.63	0.76	0.82	0.63	−0.77
PC1/R_F/RP-18	−0.65	0.70	0.54	0.65	−0.70	−0.86	0.86	0.70	0.86	−0.86	−0.83	0.84	0.53	0.83	−0.84	−0.83	0.85	0.76	0.83	−0.84
PC1/R_M/RP-18	0.69	−0.79	−0.55	−0.69	0.79	0.83	−0.89	−0.76	−0.83	0.89	0.81	−0.86	−0.42	−0.81	0.85	0.80	−0.87	−0.86	−0.80	0.87
mR_F/RP-18W	0.74	−0.77	−0.68	−0.74	0.77	0.86	−0.86	−0.81	−0.86	0.86	0.84	−0.85	−0.67	−0.84	0.85	0.84	−0.85	−0.80	−0.84	0.85
mR_M/RP-18W	−0.77	0.82	0.71	0.78	−0.82	−0.87	0.89	0.84	0.87	−0.89	−0.86	0.87	0.66	0.86	−0.88	−0.85	0.87	0.85	0.85	−0.87
R_{M0}/RP-18W	−0.79	0.80	0.72	0.79	−0.80	−0.86	0.84	0.81	0.86	−0.85	−0.84	0.84	0.73	0.84	−0.84	−0.84	0.84	0.79	0.84	−0.84
PC1/R_F/RP-18W	−0.74	0.77	0.68	0.74	−0.77	−0.86	0.86	0.80	0.86	−0.86	−0.84	0.85	0.68	0.84	−0.85	−0.84	0.84	0.80	0.84	−0.84
PC1/R_M/RP-18W	0.78	−0.82	−0.71	−0.78	0.82	0.87	−0.89	−0.84	−0.87	0.89	0.86	−0.87	−0.67	−0.86	0.88	0.85	−0.87	−0.85	−0.85	0.87
mR_F CN	0.36	−0.48	−0.20	−0.36	0.48	0.63	−0.68	−0.45	−0.63	0.68	0.59	−0.63	−0.10	−0.59	0.63	0.59	−0.65	−0.63	−0.59	0.65
mR_M CN	−0.39	0.53	0.23	0.39	−0.53	−0.62	0.69	0.49	0.62	−0.69	−0.59	0.64	0.09	0.59	−0.63	−0.58	0.66	0.68	0.58	−0.66
R_{M0} CN	−0.09	0.18	−0.07	0.09	−0.18	−0.34	0.37	0.26	0.34	−0.37	−0.30	0.32	0.00	0.30	−0.32	−0.31	0.34	0.29	0.31	−0.34
PC1/R_F/CN	−0.35	0.48	0.20	0.36	−0.48	−0.63	0.68	0.45	0.63	−0.68	−0.59	0.63	0.10	0.59	−0.62	−0.59	0.65	0.63	0.59	−0.65
PC1/R_M/CN	0.38	−0.52	−0.22	−0.38	0.52	0.61	−0.68	−0.48	−0.61	0.68	0.58	−0.63	−0.08	−0.58	0.63	0.57	−0.65	−0.67	−0.57	0.66
mR_F/Paraffin	0.93	−0.93	−0.91	−0.93	0.93	0.92	−0.93	−0.79	−0.92	0.93	0.93	−0.94	−0.70	−0.93	0.94	0.93	−0.94	−0.91	−0.93	0.94
mR_M/Paraffin	−0.92	0.93	0.90	0.92	−0.93	−0.93	0.94	0.79	0.93	−0.94	−0.94	0.95	0.67	0.94	−0.95	−0.93	0.95	0.93	0.93	−0.95
R_{M0}/Paraffin	−0.84	0.84	0.85	0.84	−0.84	−0.61	0.65	0.70	0.61	−0.65	−0.65	0.67	0.58	0.65	−0.67	−0.61	0.66	0.81	0.61	−0.67
PC1/R_F/Paraffin	−0.93	0.93	0.91	0.93	−0.93	−0.92	0.93	0.79	0.92	−0.93	−0.93	0.94	0.70	0.93	−0.94	−0.93	0.94	0.91	0.93	−0.94
PC1/R_M/Paraffin	0.92	−0.93	−0.90	−0.92	0.93	0.93	−0.94	−0.79	−0.93	0.94	0.94	−0.95	−0.67	−0.94	0.95	0.93	−0.95	−0.93	−0.93	0.95

Table 3 Correlation matrix of the lipophilicity indices obtained on RP-18, RP-18W, CN and paraffin impregnated plates

	RP-18					RP-18W					CN					Paraffin				
	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M
mR_F/RP-18	1.00	−0.94	−0.71	−1.00	0.94	0.94	−0.93	−0.90	−0.94	0.93	0.87	−0.83	−0.74	−0.87	0.83	0.64	−0.67	−0.29	−0.64	0.67
mR_M/RP-18		1.00	0.91	0.94	−1.00	−0.88	0.90	0.83	0.88	−0.90	−0.90	0.90	0.70	0.90	−0.90	−0.69	0.72	0.43	0.69	−0.72
R_{M0}/RP-18			1.00	0.71	−0.91	−0.67	0.72	0.61	0.66	−0.71	−0.80	0.84	0.55	0.80	−0.84	−0.62	0.65	0.49	0.62	−0.65
PC1/R_F/RP-18				1.00	−0.94	−0.94	0.93	0.90	0.94	−0.93	−0.87	0.83	0.74	0.87	−0.83	−0.64	0.67	0.29	0.64	−0.67
PC1/R_M/RP-18					1.00	0.88	−0.90	−0.83	−0.87	0.90	0.90	−0.90	−0.70	−0.90	0.90	0.69	−0.72	−0.43	−0.69	0.72
mR_F/RP-18W						1.00	−1.00	−0.98	−1.00	1.00	0.77	−0.75	−0.65	−0.77	0.75	0.66	−0.68	−0.49	−0.66	0.68
mR_M/RP-18W							1.00	0.96	0.99	−1.00	−0.78	0.77	0.62	0.78	−0.77	−0.70	0.72	0.55	0.70	−0.72
R_{M0}/RP-18W								1.00	0.98	−0.97	−0.66	0.63	0.57	0.66	−0.63	−0.68	0.69	0.51	0.68	−0.69
PC1/R_F/RP-18W									1.00	−0.99	−0.77	0.74	0.65	0.77	−0.74	−0.66	0.67	0.48	0.66	−0.67
PC1/R_M/RP-18W										1.00	0.77	−0.76	−0.62	−0.77	0.76	0.70	−0.72	−0.55	−0.70	0.72
mR_F CN											1.00	−0.99	−0.87	−1.00	0.99	0.38	−0.43	−0.15	−0.38	0.43
mR_M CN												1.00	0.82	0.99	−1.00	−0.41	0.46	0.24	0.41	−0.46
R_{M0} CN													1.00	0.87	−0.83	−0.02	0.06	−0.19	0.02	−0.06
PC1/R_F/CN														1.00	−0.99	−0.38	0.43	0.15	0.38	−0.43
PC1/R_M/CN															1.00	0.40	−0.45	−0.23	−0.40	0.45
mR_F/Paraffin																1.00	−1.00	−0.76	−1.00	1.00
mR_M/Paraffin																	1.00	0.75	1.00	−1.00
R_{M0}/Paraffin																		1.00	0.76	−0.76
PC1/R_F/Paraffin																			1.00	−1.00
PC1/R_M/Paraffin																				1.00

Table 4 Correlation matrix of the lipophilicity indices obtained on TOA, olive, sunflower and corn oil impregnated plates

	TOA					Olive					Sunflower				Corn					
	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M	mR_F	mR_M	R_{M0}	PC1 R_F	PC1 R_M
mR_F/TOA	1.00	−0.98	−0.96	−1.00	0.98	0.88	−0.88	−0.86	−0.88	0.88	0.89	−0.90	−0.78	−0.89	0.90	0.88	−0.89	−0.92	−0.88	0.90
mR_M/TOA		1.00	0.92	0.98	−1.00	−0.89	0.92	0.89	0.89	−0.92	−0.90	0.93	0.70	0.90	−0.93	−0.88	0.93	0.97	0.88	−0.93
R_{M0}/TOA			1.00	0.96	−0.92	−0.82	0.82	0.87	0.82	−0.82	−0.85	0.85	0.86	0.85	−0.85	−0.83	0.83	0.84	0.83	−0.83
PC1/R_F/TOA				1.00	−0.98	−0.88	0.88	0.86	0.88	−0.88	−0.89	0.90	0.78	0.89	−0.90	−0.88	0.89	0.92	0.88	−0.90
PC1/R_M/TOA					1.00	0.89	−0.92	−0.89	−0.89	0.92	0.90	−0.93	−0.70	−0.90	0.93	0.88	−0.93	−0.97	−0.88	0.93
mR_F/Olive						1.00	−0.98	−0.78	−1.00	0.98	1.00	−0.99	−0.71	−1.00	0.99	1.00	−0.99	−0.91	−1.00	0.99
mR_M/Olive							1.00	0.83	0.98	−1.00	−0.98	1.00	0.66	0.98	−1.00	−0.97	1.00	0.95	0.97	−1.00
R_{M0}/Olive								1.00	0.78	−0.84	−0.79	0.84	0.79	0.79	−0.84	−0.76	0.82	0.85	0.76	−0.83
PC1/R_F/Olive									1.00	−0.98	−1.00	0.99	0.71	1.00	−0.99	−1.00	0.99	0.91	1.00	−0.99
PC1/R_M/Olive										1.00	0.98	−1.00	−0.67	−0.98	1.00	0.97	−1.00	−0.95	−0.97	1.00
mR_F/Sunflower											1.00	−0.99	−0.74	−1.00	0.99	1.00	−0.99	−0.92	−1.00	0.99
mR_M/Sunflower												1.00	0.71	0.99	−1.00	−0.98	1.00	0.95	0.98	−1.00
R_{M0}/Sunflower													1.00	0.74	−0.72	−0.72	0.67	0.61	0.72	−0.67
PC1/R_F/Sunflower														1.00	−0.99	−1.00	0.99	0.92	1.00	−0.99
PC1/R_M/Sunflower															1.00	0.98	−1.00	−0.95	−0.98	1.00
mR_F/Corn																1.00	−0.98	−0.90	−1.00	0.98
mR_M/Corn																	1.00	0.95	0.98	−1.00
R_{M0}/Corn																		1.00	0.90	−0.96
PC1/R_F/Corn																			1.00	−0.98
PC1/R_M/Corn																				1.00

Table 5 Eigenvalues of the covariance matrix and cumulative proportion for R_F and R_M values

Principal component	R_F							
	RP-18		RP-18W		CN		Paraffin	
	Eigenvalues	Cumulative proportion	Eigenvalues	Cumulative proportion	Eigenvalues	Cumulative proportion	Eigenvalues	Cumulative proportion
1	0.110557	99.91	0.105039	98.59	0.134812	96.30	0.353480	99.49
2	0.000078	99.98	0.001290	99.80	0.004765	99.70	0.001722	99.98
3	0.000011	99.99	0.000171	99.96	0.000229	99.87	0.000076	100.00
4	0.000005	99.99	0.000025	99.99	0.000104	99.94	0.000011	100.00
5	0.000003	100.00	0.000015	100.00	0.000083	100.00		
	TOA		Olive		Sunflower		Corn	
	Eigenvalues	Cumulative proportion	Eigenvalues	Cumulative proportion	Eigenvalues	Cumulative proportion	Eigenvalues	Cumulative proportion
1	0.372598	99.60	0.287769	99.15	0.313127	99.00	0.286996	99.17
2	0.001369	99.96	0.002116	99.88	0.003045	99.97	0.002248	99.94
3	0.000104	99.99	0.000237	99.96	0.000080	99.99	0.000132	99.99
4	0.000035	100.00	0.000084	99.99	0.000017	100.00	0.000026	100.00
5			0.000040	100.00	0.000005	100.00	0.000009	100.00
	R_M							
	RP-18		RP-18W		CN		Paraffin	
	Eigenvalues	Cumulative proportion	Eigenvalues	Cumulative proportion	Eigenvalues	Cumulative proportion	Eigenvalues	Cumulative proportion
1	1.146627	99.47	0.501052	99.17	0.815962	97.88	1.667825	99.55
2	0.005744	99.97	0.002571	99.68	0.016176	99.82	0.006345	99.93
3	0.000229	99.99	0.001430	99.96	0.000888	99.92	0.000994	99.99
4	0.000057	100.00	0.000128	99.98	0.000390	99.97	0.000143	100.00
5	0.000016	100.00	0.000084	100.00	0.000249	100.00		
	TOA		Olive		Sunflower		Corn	
	Eigenvalues	Cumulative proportion	Eigenvalues	Cumulative proportion	Eigenvalues	Cumulative proportion	Eigenvalues	Cumulative proportion
1	3.068815	99.80	1.819938	98.92	1.809411	98.54	1.936167	99.60
2	0.004954	99.96	0.016554	99.82	0.026051	99.95	0.006727	99.95
3	0.001168	100.00	0.002438	99.95	0.000592	99.99	0.000493	99.98
4	0.000066	100.00	0.000628	99.98	0.000201	100.00	0.000378	100.00
5			0.000326	100.00	0.000053	100.00	0.000089	100.00

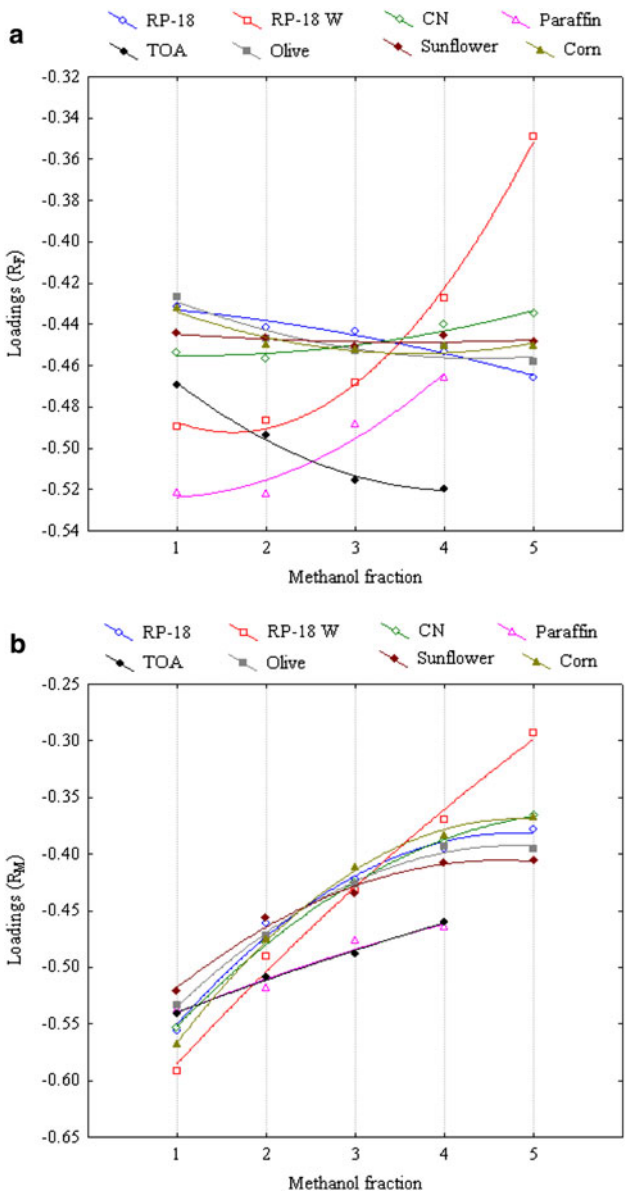

Fig. 2 Profiles of loadings corresponding to R_F values (**a**) and R_M values (**b**)

the bottom and 0.7 cm from the sides. The distance between the spots was 0.7 cm. The elution was performed by ascendant development in a chromatography chamber previously saturated for 10 min. All the plates were developed through a distance of 8 cm. Three types of chemically bonded stationary phases were used alongside five other types of oil impregnated plates. The chemically bonded plates were made of RP-18 silica gel 60 modified with aliphatic hydrocarbons of increasing chain length resulting in increased hydrophobic. The special HPTLC RP-18W plates with a defined lower degree of surface modification can be wetted and developed with pure water. The CN-modified plates, based on silica gel 60 are altered by cyanopropyl groups. The silica gel 60 F_{254} plates were impregnated with 10% oil solution in diethyl ether. The impregnation was performed by ascendant development with oil solutions.

Each type of stationary phase were eluted with four (TOA and paraffin) or five (the rest of the stationary phases) mobile phases containing different mixtures of methanol and water, which were optimized in order to obtain a significant amount of migration while the mobile phase composition was modified. The methanol ranges used in the mobile phases were 95–99% for RP-18 (changed with 1% per step), 89–97% for RP-18W (changed with 2% per step), 71–83% for CN (changed with 3% per step), 90–94 for paraffin (changed with 1% per step), 92–98% for TOA (changed with 2% per step) and, 90–98% for olive, sunflower and corn oil impregnated plates (changes with 2% per step). The R_F values in duplicate runs were calculated for each step.

Results and Discussion

The lipophilicity indices estimated from chromatographic results obtained from the reversed-phases investigated are shown in Table 1. The regression correlation coefficients between R_M and C (Eq. 1) indicated a very good linearity through the concentration of methanol used as the organic modifier. The correlation coefficient (r) presented values higher then 0.98 in all cases, excepting lutein ($r_{TOA} = 0.97$, $r_{Olive} = 0.97$), γ-tocopherol ($r_{Olive} = 0.97$, $r_{Sunflower} = 0.96$) and δ-tocopherol ($r_{Corn} = 0.97$). In addition, the retention profiles of the compounds presented in Fig. 1a–h support the chromatographic regularities mentioned above and illustrate in a good way the (dis)similarities between the investigated stationary phases. For example a good correlation is easily observed between vegetable oils. Similar behavior is observed between paraffin oil and TOA. These patterns are also in good agreement with the results of the correlation matrices shown in Tables 2–4.

In nearly all cases the results shown in Table 1 reveal that β-carotene and lycopene have the highest lipophilicity, followed by the tocopherols and xanthophylls. The least lipophilic compounds were the retinoids. However, we have to comment on an unexpected R_{M0} value for retinol derived from using the sample on paraffin oil impregnated plates. This discrepancy may be explained by the migration of the spots near the front of solvent. In this case, the errors are larger and proportional to the concentration of methanol, which is also known as the heteroscedasticity effect [25]. A direct consequence of this effect is the overestimated value of the intercept corresponding to 0% methanol. For instance, by comparing data in Table 1 one may

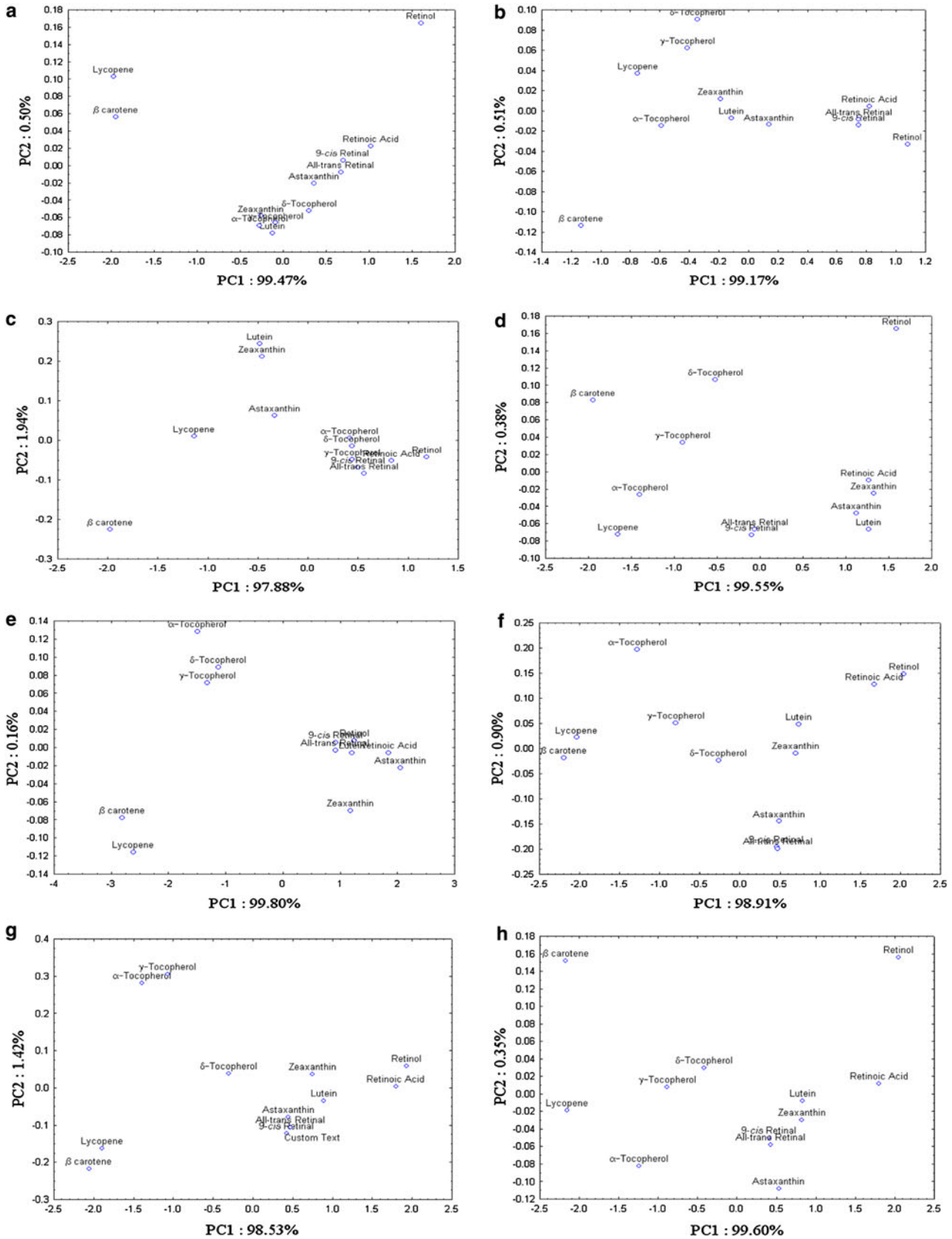

Fig. 3 Lipophilicity charts corresponding to R_M: RP-18 (**a**); RP-18W (**b**); CN (**c**); paraffin (**d**); TOA (**e**); olive (**f**); sunflower (**g**); corn (**h**)

observe that the differences between arithmetic means and scores of retinol ($mR_F = 0.867$; $mR_M = -0.837$; $R_{M0} = 12.487$; PC1/$R_M = -0.693$; PC1/$R_M = 1.591$) and the corresponding values of its congeners, zeaxanthin ($mR_F = 0.832$; $mR_M = -0.699$; $R_{M0} = 5.508$; PC1/$R_M = -0.624$; PC1/$R_M = 1.325$) and retinoic acid ($mR_F = 0.823$; $mR_M = -0.672$; $R_{M0} = 6.410$; PC1/$R_M = -0.604$; PC1/$R_M = 1.270$) are not so large. Furthermore, the lipophilicity indices may be used to characterize and compare the reverse stationary phases concerning their lipophilic character. From these findings we may conclude that the RP-18 plates followed by the RP-18W are the most lipophilic reversed-phases and the CN stationary phase is the least lipophilic. Concerning the vegetable oils, all the lipophilicity indices indicate that corn oil is the most lipophilic, closely followed by the olive and sunflower oils.

Applying PCA on the data matrix corresponding to R_F values (12 compounds and five or four solvent mixtures) it was found that the first principal component accounts for more than 99% of the total variance (information) in the retention data in all cases with the exception of RP-18W (98.59%) and CN (96.30%); in all cases, the first two principal components account for 99.68% of the total variance (Table 5). At the same time, the plot of loadings (contribution of the solvent mixture to the dispersion of spots) as a function of the methanol content for all stationary phases (Fig. 2a–b) reveals once again a high similarity within some groups of the investigated stationary phases: vegetable oils impregnated-plates and RP-18 in the first group (linear profiles of R_F) and RP-18W, paraffin oil and TOA, in the second group (quadratic profile of R_F). Figure 3a and b are complementary and point out a retention gradient induced by increasing the methanol content in the mobile phase. All the statements above are well supported by lipophilicity charts obtained by scatterplot of scores corresponding to R_M values onto the planes described by the first two principal components as is illustrated in Fig. 3a–h. The RP-18 stationary phase groups the compounds in two clusters, while a better separation can be observed in the case of RP-18W plates. The patterns corresponding to the RP-18W, TOA and paraffin plates present high similarities with those obtained on vegetable oils impregnated-plates.

Conclusions

The lipophilicity indices of some lipophilic vitamins and their precursors on RP-18, RP-18W, CN and silica gel layers impregnated with a variety of oils including vegetable oils (olive, sunflower and corn oil) and synthetic oils (trioctylamine and paraffin oil) were determined using water–methanol as the mobile phase. In most cases a highly significant correlation was found between the R_M values and the methanol concentration in the eluent. The results obtained allow a pertinent evaluation of the lipophilic character of the oils and their objective ranking. In addition, the scores plots provide the lipophilicity charts of investigated compounds and loadings plots afford a deeper insight into the complex chromatographic mechanism. This investigative approach might be extended to other vegetable and synthetic oils and fats because their lipophilicity is involved in various biological and technical processes.

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