Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Lipophilicity of Flavonoids Estimated by Reversed-Phase High Performance Thin-Layer Chromatography: Chemically Bonded Plates vs. Impregnated Plates with Oils, Animal, and Human Fats

Rodica Domnica Briciu[a]; Costel Sârbu[a]
[a] Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University, Cluj Napoca, Romania

Online publication date: 02 June 2010

To cite this Article Briciu, Rodica Domnica and Sârbu, Costel(2010) 'Lipophilicity of Flavonoids Estimated by Reversed-Phase High Performance Thin-Layer Chromatography: Chemically Bonded Plates vs. Impregnated Plates with Oils, Animal, and Human Fats', Separation Science and Technology, 45: 9, 1275 — 1285
To link to this Article: DOI: 10.1080/01496391003775907
URL: http://dx.doi.org/10.1080/01496391003775907

Separation Science and Technology, 45: 1275–1285, 2010

ISSN: 0149-6395 print/1520-5754 online
DOI: 10.1080/01496391003775907

Taylor & Francis
Taylor & Francis Group

Lipophilicity of Flavonoids Estimated by Reversed-Phase High Performance Thin-Layer Chromatography: Chemically Bonded Plates vs. Impregnated Plates with Oils, Animal, and Human Fats

Rodica Domnica Briciu and Costel Sârbu
Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University, Cluj Napoca, Romania

Silica gel plates impregnated with a variety of oils (olive oil, sunflower oil, corn oil, trioctylamine, and paraffin oil) and fats (margarine, butter, cod liver fat, pig fat, sheep fat, pullet fat, and human fat) were evaluated and compared with the commercially available reversed-phases TLC plates (RP-18, RP-18 W, and CN). A representative series of flavonoids is employed to evaluate the suitability of oils and fats as reversed-phases for TLC and to provide different lipophilicity indices: R_{M0}, scores corresponding to first principal component of R_F and/or R_M, 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 ($r > 0.96$) and allowed for accurate estimation of lipophilicity of selected flavonoids and to ranking the lipophilicity of oils and fats when comparing with chemically bonded phases. The human fat-impregnated plates provided lipophilicity values closely associated with those obtained for margarine and butter. Moreover, the human fat lipophilic character seems to be placed between pullet and cod fats. Concerning the lipophilicity scale of vegetable oils, it is worth noting that the corn oil presents the highest lipophilicity, closely followed by the sunflower and olive oils.

Keywords animal fats; human fat; lipophilicity; PCA; RP-TLC; vegetable oils

INTRODUCTION

Each year an increasing number of bioactive compounds with different therapeutic effects are synthesized and introduced on the market. In many cases the biological and biochemical effects are based on their physicochemical properties such as lipophilicity or solubility. The lipophilicity has been defined by IUPAC as the affinity of a molecule for a lipophilic environment and it is measured by its distribution behavior in a biphasic system either liquid-liquid or solid-liquid (1). This particular property is usually associated with an increased biological activity, poorer aqueous solubility, faster metabolization and elimination, increased plasma protein binding, sometimes shorter duration of action. It plays also an important role in the pharmacodynamic and toxicological profile of drugs (2,3). Lipophilicity is usually expressed by the partition coefficient, denoted in few different ways, frequently depending on the determination method (Log P, Log k_w, Log K_{ow}). However, the real importance of the lipophilicity has been defined within the Quantitative Structure-Activity Relationship (QSAR), Quantitative Structure-Retention Relationship (QSRR), or Quantitative Structure-Property Relationship (QSPR) approaches (4). Over Hansch (5), the lipophilic character and at the same time the biological activity of compounds are strongly influenced by the molecular substituents. A similar effect may be caused by the environment in which the molecule is actually placed. For predicting a given physicochemical or biological property the relationship between the chemical structure of the investigated compounds and the desired property must be identified. Furthermore, the goals of the QSAR tests are to create a realistic insight into the ability of a compound to cross the fat biological barriers formed by the cellular membrane.

Determination of the partition coefficient by the equilibration method using the 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 cost, and easy to perform. In addition, we have to stress the dynamic aspect of the chromatographic process

Received 12 June 2009; accepted 4 February 2010.
Address correspondence to Costel Sârbu, Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University, Arany Janos Str. No. 11, Cluj Napoca, RO-400028, Romania. Tel.: +40-264-593833; Fax: +40-264-590818. E-mail: csarbu@chem.ubbcluj.ro

and the wide choice of stationary phases and developing solvents. A lot of lipophilicity studies were based on RP-18 stationary phases 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 and fats (more or less similar with the lipidic biological membranes) might be one of the most realistic alternatives. Over the years, the paraffin oil (8–12) nearby silicon oil (13,14) and ethyl oleate (15) were often used for silica gel plates impregnation in order to change the stationary phase characteristics. The chemical composition of the vegetable oils and derived products indicates a high amount of triglycerides, free fatty acids (especially oleic and linoleic acid), phytosterols, lipophilic vitamins, and traces of minerals (16,17). On the other side, the animal fats present a high concentration of saturated fatty acids and cholesterol (18).

The purpose of this work is to investigate the feasibility of silica gel plates impregnated with a variety of oils and fats and to compare them with the commercially available reversed-phases HPTLC plates. In this order, the lipophilicity of some flavonoids has been determined using mobile phases containing various concentrations of methanol in water and estimated by different indices. In addition, the scores obtained applying the Principal Component Analysis (PCA) offer the possibility to get a new lipophilcity scale and the lipophilcity chart of compounds and the reversed-phases investigated; eigenvalues and eigenvectors (loadings) giving new insights about the chromatographic mechanism and the behavior of compounds.

THEORY

RP-HPTLC provides a variety of indices (descriptors) that can be used as lipophilicity estimators (19,20). The most popular lipophilicity indices measured by RP-HPTLC are derived by the retention R_F values according to the linear relationship described by the Soczewiński-Wachtmeister equation:

$$R_M = R_{M0} + bC, \tag{1}$$

where R_M is defined by Bate-Smith and Westall (21) through the following formula

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

The R_{M0} indicates the extrapolated value to the pure water as mobile phase and it is the HPTLC descriptor most frequently used into 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 an alternative descriptor of lipophilicity. 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 the lipophilicity indices (22). It is also possible to obtain a new lipophilicity scale applying PCA directly to the matrix of retention data resulted for all compounds and combinations of methanol-water (R_F and/or R_M values obtained for all values of C). 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). In addition, a careful investigation of eigenvalues and eigenvectors (loadings) can offer useful information concerning the chromatographic behavior of the compounds and the retention mechanism (23–27). All these lipophilicity descriptors and graphs are computed through the Statistica 7.0 program (28).

EXPERIMENTAL

All the compounds and solvents were obtained from a commercial source (Merck, Fluka, and Sigma) in analytical degree purity. The oils (paraffin, trioctylamine-TOA, olive, sunflower, corn, cod liver) and fats (margarine, butter, pig, sheep, pullet) used for the impregnation were from the local market; woman fat was obtained from liposuction surgery. The standard solutions of flavonoids (1: flavone; 2: 2′-methoxyflavone; 3: 3-methoxyflavone; 4: 5-methoxyflavone; 5: 6-methoxyflavone; 6: 7-methoxyflavone; 7: 7,8-dimethoxyflavone; 8: 3-hydroxy-7-methoxyflavone; 9: 3-hydroxyflavone; 10: 5-hydroxyflavone; 11: 6-hydroxyflavone; 12: 7-hydroxyflavone; 13: 3,6-dihydroxyflavone; 14: 3,7-dihydroxyflavone; 15: chrysine; 16: apigenin; 17: baicalein; 18: galangin; 19: kaempferol; 20: luteolin; 21: quercetin; 22: fisetin; 23: geraldol; 24: 6-methylflavone; 25: 6-chloro-7-methylflavone; 26: daidzein) were prepared in methanol (1 mg mL^{-1}). The spots (1 μL) were applied at 1.5 cm from bottom edge and at 0.7 cm from lateral edges using a Hamilton microsyringe of 10 μL. The distance between the spots was by 0.7 cm. The elution was performed by ascendant development into a chromatographic chamber previously saturated for 10 minutes. Three types of chemically bonded stationary phases were used near by other twelve types of fat-impregnated plates. The chemically bonded plates were by RP-18 silica gel 60 modified with aliphatic hydrocarbons of increasing hydrocarbon chain length resulting in increased hydrophobic, the special HPTLC RP-18 W with a defined lower degree of surface modification can be wetted and developed even with pure water and the CN-modified plate which are based on a silica gel 60 modified with cyanopropyl groups. The silica gel 60 F_{254} plates (10 × 20 cm) were impregnated with 10%

TABLE 1
The lipophilicity indices obtained on chemically bonded (RP-18, RP-18 W, CN) and paraffin-impregnated plates

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
Flavone	0.215	0.616	3.667	0.226	−0.450	0.271	0.454	2.400	0.013	0.020	0.334	0.313	1.873	−0.059	0.161	0.347	0.297	2.381	0.247	−0.432
2′-methoxyflavone	0.187	0.706	3.991	0.290	−0.654	0.237	0.541	2.650	0.089	−0.178	0.314	0.357	2.053	−0.014	0.061	0.324	0.363	2.969	0.304	−0.609
3-methoxyflavone	0.199	0.664	3.799	0.262	−0.559	0.248	0.513	2.607	0.065	−0.117	0.324	0.335	2.007	−0.037	0.111	0.305	0.395	2.804	0.341	−0.666
5-methoxyflavone	0.194	0.670	3.576	0.274	−0.571	0.235	0.542	2.559	0.092	−0.179	0.370	0.241	1.757	−0.141	0.324	0.414	0.181	3.356	0.122	−0.241
6-methoxyflavone	0.143	0.858	4.228	0.390	−0.996	0.209	0.613	2.697	0.151	−0.338	0.313	0.367	2.449	−0.013	0.033	0.349	0.334	3.950	0.269	−0.600
7-methoxyflavone	0.175	0.736	3.855	0.317	−0.719	0.214	0.598	2.608	0.140	−0.302	0.296	0.396	2.151	0.025	−0.028	0.370	0.275	3.511	0.218	−0.451
7,8-dimethoxyflavone	0.209	0.629	3.593	0.240	−0.480	0.236	0.537	2.401	0.091	−0.162	0.332	0.320	2.064	−0.055	0.142	0.410	0.190	3.339	0.129	−0.259
3-hydroxy-7-methoxyflavone	0.144	0.850	4.141	0.387	−0.978	0.159	0.777	3.165	0.262	−0.711	0.192	0.641	1.963	0.260	−0.568	0.274	0.482	3.315	0.413	−0.879
3-hydroxyflavone	0.164	0.771	3.812	0.343	−0.796	0.204	0.633	2.945	0.161	−0.391	0.258	0.486	2.331	0.112	−0.231	0.306	0.399	3.076	0.347	−0.685
5-hydroxyflavone	0.122	0.941	4.313	0.437	−1.180	0.194	0.666	3.035	0.184	−0.464	0.207	0.605	2.116	0.226	−0.489	0.241	0.562	3.408	0.486	−1.049
6-hydroxyflavone	0.327	0.338	2.957	−0.026	0.176	0.315	0.349	1.850	−0.087	0.265	0.388	0.209	1.955	−0.181	0.390	0.513	−0.030	2.734	−0.103	0.247
7-hydroxyflavone	0.353	0.284	2.789	−0.083	0.298	0.311	0.360	2.002	−0.078	0.235	0.391	0.206	2.198	−0.189	0.393	0.718	−0.416	0.798	−0.591	1.175
3,6-dihydroxyflavone	0.294	0.419	3.461	0.047	−0.011	0.287	0.413	2.167	−0.025	0.116	0.324	0.341	2.273	−0.038	0.093	0.443	0.113	3.108	0.056	−0.080
3,7-dihydroxyflavone	0.292	0.417	3.168	0.054	−0.004	0.263	0.478	2.579	0.031	−0.039	0.299	0.399	2.478	0.018	−0.039	0.585	−0.158	1.613	−0.281	0.581
Chrysine	0.273	0.462	3.178	0.096	−0.104	0.271	0.472	3.087	0.014	−0.042	0.219	0.566	1.820	0.200	−0.399	0.644	−0.266	1.110	−0.423	0.839
Apigenin	0.463	0.070	2.481	−0.332	0.776	0.320	0.353	2.576	−0.095	0.235	0.268	0.461	2.243	0.088	−0.174	0.740	−0.463	0.605	−0.644	1.286
Baicalein	0.315	0.354	2.447	0.002	0.147	0.282	0.427	2.282	−0.012	0.082	0.319	0.346	2.003	−0.025	0.086	0.540	−0.071	0.834	−0.199	0.440
Galangin	0.255	0.507	3.386	0.135	−0.205	0.239	0.545	2.970	0.085	−0.198	0.171	0.714	2.341	0.307	−0.736	0.555	−0.105	1.881	−0.210	0.455
Kaempferol	0.442	0.107	2.321	−0.282	0.695	0.296	0.396	2.253	−0.044	0.150	0.252	0.502	2.436	0.124	−0.268	0.719	−0.434	1.464	−0.583	1.177
Luteolin	0.559	−0.108	1.870	−0.544	1.178	0.286	0.416	2.237	−0.022	0.106	0.312	0.361	2.067	−0.010	0.051	0.346	0.278	0.645	0.218	−0.290
Quercetin	0.493	0.014	2.273	−0.396	0.903	0.277	0.427	1.677	−0.003	0.100	0.277	0.431	1.786	0.070	−0.100	0.351	0.267	0.537	0.204	−0.261
Fisetin	0.553	−0.097	1.667	−0.530	1.158	0.287	0.405	1.668	−0.025	0.147	0.312	0.350	1.403	−0.009	0.086	0.598	−0.176	0.824	−0.327	0.666
Geraldol	0.438	0.119	2.951	−0.277	0.661	0.210	0.589	1.931	0.145	−0.263	0.219	0.565	1.802	0.199	−0.397	0.584	−0.160	1.866	−0.273	0.574
6-methylflavone	0.140	0.875	4.408	0.395	−1.036	0.197	0.660	3.134	0.178	−0.455	0.249	0.503	2.190	0.131	−0.265	0.296	0.444	3.711	0.375	−0.819
6-chloro-7-methylflavone	0.081	1.171	4.926	0.530	−1.698	0.139	0.867	3.680	0.306	−0.923	0.165	0.747	2.769	0.320	−0.816	0.192	0.753	4.471	0.597	−1.518
Daidzein	0.639	−0.263	2.051	−0.723	1.521	0.487	0.024	2.000	−0.469	0.971	0.479	0.039	1.540	−0.384	0.774	0.761	−0.509	0.400	−0.693	1.398

TABLE 2
The lipophilicity indices obtained on TOA, olive, sunflower and corn-impregnated plates

	TOA					Olive					Sunflower					Corn				
Compound	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
Flavone	0.579	−0.142	1.023	−0.360	0.833	0.322	0.349	2.391	0.209	−0.374	0.234	0.530	1.838	0.106	−0.126	0.307	0.377	2.360	0.192	−0.319
2′-methoxyflavone	0.596	−0.172	0.743	−0.405	0.922	0.330	0.334	2.501	0.194	−0.347	0.212	0.587	1.938	0.156	−0.255	0.317	0.365	2.649	0.171	−0.298
3-methoxyflavone	0.470	0.053	1.428	−0.114	0.416	0.287	0.424	2.559	0.286	−0.544	0.191	0.641	1.840	0.205	−0.375	0.277	0.451	2.619	0.259	−0.487
5-methoxyflavone	0.761	−0.508	0.374	−0.772	1.624	0.400	0.193	2.489	0.041	−0.041	0.307	0.375	2.205	−0.064	0.214	0.405	0.180	2.262	−0.027	0.118
6-methoxyflavone	0.567	−0.120	0.928	−0.337	0.797	0.274	0.457	2.674	0.315	−0.621	0.158	0.740	1.858	0.280	−0.596	0.258	0.497	2.728	0.301	−0.591
7-methoxyflavone	0.655	−0.282	0.562	−0.539	1.152	0.320	0.352	2.442	0.214	−0.382	0.185	0.671	2.295	0.215	−0.446	0.332	0.317	1.935	0.135	−0.177
7,8-dimethoxyflavone	0.750	−0.480	0.137	−0.754	1.590	0.364	0.258	2.167	0.117	−0.167	0.248	0.510	2.414	0.068	−0.091	0.357	0.269	2.031	0.080	−0.074
3-hydroxy-7-methoxyflavone	0.248	0.558	3.615	0.397	−0.792	0.173	0.732	2.996	0.534	−1.230	0.109	0.955	2.811	0.388	−1.082	0.126	0.867	2.351	0.596	−1.400
3-hydroxyflavone	0.250	0.535	3.233	0.383	−0.720	0.210	0.617	2.756	0.452	−0.970	0.116	0.914	2.586	0.372	−0.990	0.148	0.779	2.062	0.546	−1.199
5-hydroxyflavone	0.259	0.507	3.037	0.360	−0.649	0.144	0.819	2.801	0.595	−1.410	0.079	1.095	2.518	0.459	−1.391	0.098	0.987	2.317	0.658	−1.663
6-hydroxyflavone	0.572	−0.127	0.278	−0.369	0.876	0.490	0.019	1.957	−0.163	0.359	0.307	0.368	1.840	−0.060	0.233	0.418	0.146	1.218	−0.057	0.215
7-hydroxyflavone	0.675	−0.323	0.687	−0.577	1.223	0.715	−0.401	0.242	−0.682	1.341	0.454	0.082	1.207	−0.386	0.877	0.748	−0.475	0.185	−0.795	1.610
3,6-dihydroxyflavone	0.275	0.519	4.074	0.340	−0.785	0.353	0.282	2.263	0.139	−0.224	0.211	0.593	2.084	0.157	−0.270	0.291	0.395	1.514	0.226	−0.339
3,7-dihydroxyflavone	0.325	0.335	2.042	0.206	−0.209	0.435	0.117	1.428	−0.049	0.170	0.290	0.395	1.456	−0.019	0.177	0.512	−0.021	1.232	−0.268	0.584
Chrysine	0.404	0.208	3.110	0.064	−0.078	0.572	−0.127	0.560	−0.363	0.734	0.309	0.358	1.479	−0.060	0.259	0.549	−0.088	1.241	−0.351	0.733
Apigenin	0.331	0.479	5.869	0.257	−0.886	0.727	−0.436	0.813	−0.702	1.391	0.447	0.095	1.364	−0.372	0.844	0.700	−0.375	0.675	−0.688	1.378
Baicalein	0.346	0.286	1.662	0.152	−0.076	0.578	−0.140	0.865	−0.372	0.748	0.450	0.090	1.426	−0.379	0.856	0.482	0.031	0.858	−0.201	0.476
Galangin	0.169	0.911	5.779	0.560	−1.734	0.379	0.221	1.586	0.076	−0.063	0.196	0.628	1.856	0.194	−0.346	0.382	0.217	1.760	0.023	0.047
Kaempferol	0.302	0.489	5.076	0.314	−0.804	0.591	−0.161	0.727	−0.401	0.800	0.356	0.265	1.645	−0.171	0.463	0.660	−0.325	2.211	−0.596	1.234
Luteolin	0.226	0.691	4.916	0.446	−1.211	0.304	0.364	1.240	0.234	−0.358	0.359	0.270	2.288	−0.185	0.446	0.324	0.321	0.884	0.153	−0.164
Quercetin	0.212	0.615	2.838	0.450	−0.838	0.394	0.191	1.242	0.038	0.017	0.353	0.281	2.190	−0.170	0.424	0.352	0.268	0.996	0.091	−0.048
Fisetin	0.264	0.587	4.897	0.373	−1.004	0.616	−0.205	0.188	−0.464	0.917	0.492	0.015	1.266	−0.473	1.024	0.636	−0.244	0.367	−0.545	1.096
Geraldol	0.375	0.324	4.697	0.150	−0.469	0.577	−0.135	0.610	−0.372	0.749	0.393	0.195	1.562	−0.253	0.620	0.514	−0.026	1.820	−0.270	0.583
6-methylflavone	0.532	−0.059	1.553	−0.243	0.618	0.267	0.474	2.608	0.329	−0.655	0.179	0.687	2.259	0.229	−0.481	0.193	0.662	2.690	0.447	−0.955
6-chloro-7-methylflavone	0.310	0.388	2.859	0.254	−0.398	0.141	0.841	3.089	0.602	−1.470	0.077	1.115	2.729	0.463	−1.437	0.097	1.059	3.682	0.662	−1.852
Daidzein	0.531	−0.058	1.731	−0.235	0.602	0.772	−0.534	0.287	−0.808	1.628	0.597	−0.173	0.549	−0.698	1.449	0.724	−0.420	−0.138	−0.742	1.494

TABLE 3
The lipophilicity indices obtained on margarine, butter and cod liver-impregnated plates

Compound	Margarine					Butter					Cod Liver				
	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}	$PC1/R_F$	$PC1/R_M$
Flavone	0.391	0.207	2.319	−0.095	0.271	0.390	0.219	2.680	−0.113	0.291	0.320	0.361	2.690	−0.012	0.068
2′-methoxyflavone	0.381	0.234	2.748	−0.074	0.199	0.384	0.234	2.897	−0.102	0.257	0.299	0.409	2.795	0.036	−0.040
3-methoxyflavone	0.363	0.268	2.720	−0.034	0.126	0.357	0.281	2.624	−0.038	0.156	0.272	0.464	2.643	0.098	−0.159
5-methoxyflavone	0.427	0.139	2.479	−0.177	0.414	0.453	0.091	2.558	−0.256	0.580	0.344	0.306	2.404	−0.065	0.194
6-methoxyflavone	0.334	0.337	3.042	0.030	−0.036	0.335	0.332	2.893	0.009	0.042	0.240	0.543	2.757	0.169	−0.337
7-methoxyflavone	0.380	0.235	2.759	−0.073	0.198	0.387	0.224	2.798	−0.108	0.282	0.285	0.425	2.295	0.069	−0.067
7,8-dimethoxyflavone	0.434	0.124	2.386	−0.192	0.449	0.430	0.143	2.903	−0.209	0.462	0.336	0.318	2.241	−0.046	0.171
3-hydroxy-7-methoxyflavone	0.190	0.685	3.055	0.359	−0.801	0.171	0.753	3.338	0.388	−0.903	0.139	0.844	3.012	0.396	−1.008
3-hydroxyflavone	0.209	0.622	2.783	0.317	−0.654	0.209	0.640	3.240	0.299	−0.646	0.163	0.747	2.583	0.344	−0.786
5-hydroxyflavone	0.233	0.588	3.451	0.259	−0.601	0.198	0.671	3.236	0.327	−0.719	0.137	0.841	2.712	0.403	−0.996
6-hydroxyflavone	0.520	−0.039	2.103	−0.382	0.814	0.534	−0.066	2.316	−0.434	0.934	0.412	0.166	2.007	−0.217	0.512
7-hydroxyflavone	0.589	−0.164	1.456	−0.531	1.107	0.629	−0.237	1.138	−0.628	1.318	0.554	−0.095	0.468	−0.530	1.112
3,6-dihydroxyflavone	0.334	0.323	2.468	0.034	0.011	0.343	0.319	2.950	−0.009	0.068	0.282	0.438	2.506	0.075	−0.100
3,7-dihydroxyflavone	0.341	0.294	1.491	0.026	0.102	0.313	0.367	2.429	0.066	−0.035	0.338	0.308	1.934	−0.050	0.197
Chrysine	0.488	0.023	1.813	−0.308	0.686	0.437	0.117	1.825	−0.207	0.526	0.403	0.181	1.890	−0.196	0.479
Apigenin	0.588	−0.159	1.044	−0.527	1.108	0.602	−0.192	1.701	−0.577	1.215	0.523	−0.041	1.424	−0.464	0.981
Baicalein	0.144	0.967	5.235	0.460	−1.483	0.115	0.953	3.298	0.523	−1.350	0.234	0.610	3.903	0.180	−0.502
Galangin	0.338	0.310	2.156	0.028	0.048	0.315	0.366	2.512	0.060	−0.034	0.272	0.451	2.221	0.097	−0.125
Kaempferol	0.498	0.004	1.580	−0.328	0.736	0.443	0.107	2.235	−0.227	0.546	0.261	0.466	1.830	0.123	−0.150
Luteolin	0.103	0.984	2.825	0.562	−1.454	0.092	1.020	2.496	0.578	−1.492	0.100	0.959	1.543	0.487	−1.240
Quercetin	0.163	0.726	1.859	0.428	−0.857	0.133	0.857	2.815	0.481	−1.133	0.262	0.513	3.384	0.117	−0.280
Fisetin	0.232	0.540	2.038	0.270	−0.455	0.192	0.646	2.125	0.350	−0.657	0.223	0.549	1.354	0.212	−0.331
Geraldol	0.272	0.446	1.974	0.179	−0.249	0.246	0.535	2.999	0.215	−0.413	0.319	0.338	1.515	−0.006	0.138
6-methylflavone	0.297	0.417	3.038	0.114	−0.210	0.309	0.400	3.206	0.068	−0.113	0.231	0.578	−0.020	0.187	−0.421
6-chloro-7-methylflavone	0.185	0.742	3.879	0.369	−0.948	0.177	0.768	3.967	0.370	−0.940	0.130	0.899	3.360	0.417	−1.135
Daidzein	0.673	−0.329	1.206	−0.717	1.478	0.717	−0.436	1.739	−0.828	1.761	0.637	−0.255	1.378	−0.718	1.457

TABLE 4
The lipophilicity indices obtained on human, pig, sheep and pullet fat-impregnated plates

Compound	Human					Pig					Sheep					Pullet				
	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
Flavone	0.370	0.255	2.485	−0.009	0.111	0.498	0.006	2.037	−0.150	0.311	0.497	0.007	1.976	−0.201	0.453	0.480	0.038	2.173	−0.110	0.217
2′-methoxyflavone	0.392	0.191	0.581	−0.076	0.385	0.471	0.054	2.271	−0.092	0.205	0.489	0.023	2.241	−0.185	0.413	0.454	0.090	2.572	−0.052	0.104
3-methoxyflavone	0.344	0.306	2.440	0.046	0.001	0.449	0.096	2.249	−0.041	0.112	0.476	0.046	2.137	−0.155	0.363	0.434	0.126	2.509	−0.007	0.024
5-methoxyflavone	0.448	0.104	2.699	−0.179	0.406	0.502	−0.004	1.972	−0.159	0.334	0.539	−0.070	1.798	−0.295	0.624	0.486	0.027	2.328	−0.123	0.245
6-methoxyflavone	0.328	0.349	2.910	0.085	−0.118	0.416	0.160	2.515	0.032	−0.030	0.435	0.123	2.298	−0.064	0.192	0.400	0.194	2.760	0.068	−0.127
7-methoxyflavone	0.387	0.229	2.881	−0.042	0.135	0.465	0.065	2.265	−0.078	0.181	0.480	0.037	1.986	−0.164	0.386	0.445	0.105	2.515	−0.032	0.072
7,8-dimethoxyflavone	0.429	0.141	2.724	−0.137	0.327	0.500	0.001	2.156	−0.155	0.323	0.532	−0.059	1.816	−0.280	0.598	0.490	0.019	2.209	−0.132	0.262
3-hydroxy-7-methoxyflavone	0.177	0.723	3.032	0.413	−0.904	0.239	0.537	2.708	0.430	−0.875	0.157	0.755	2.417	0.565	−1.216	0.273	0.466	2.988	0.354	−0.737
3-hydroxyflavone	0.214	0.608	2.754	0.331	−0.645	0.262	0.483	2.769	0.377	−0.754	0.231	0.538	1.954	0.400	−0.730	0.290	0.426	2.929	0.315	−0.649
5-hydroxyflavone	0.207	0.649	3.280	0.350	−0.767	0.296	0.415	3.150	0.299	−0.600	0.329	0.339	2.766	0.175	−0.299	0.279	0.445	2.805	0.340	−0.690
6-hydroxyflavone	0.532	−0.061	2.550	−0.365	0.763	0.599	−0.188	2.095	−0.377	0.745	0.625	−0.230	1.373	−0.484	0.985	0.574	−0.137	1.892	−0.319	0.610
7-hydroxyflavone	0.605	−0.189	1.024	−0.541	1.143	0.700	−0.370	0.274	−0.596	1.149	0.717	−0.406	0.272	−0.683	1.388	0.696	−0.366	0.759	−0.590	1.114
3,6-dihydroxyflavone	0.359	0.281	2.694	0.017	0.037	0.459	0.078	2.366	−0.066	0.151	0.361	0.254	1.386	0.110	−0.091	0.449	0.095	2.237	−0.040	0.092
3,7-dihydroxyflavone	0.323	0.349	2.493	0.093	−0.082	0.436	0.116	1.437	−0.010	0.065	0.344	0.282	0.905	0.149	−0.147	0.436	0.118	1.892	−0.009	0.039
Chrysine	0.449	0.091	1.386	−0.194	0.534	0.568	−0.121	1.120	−0.304	0.594	0.598	−0.174	0.610	−0.419	0.870	0.559	−0.105	1.169	−0.284	0.534
Apigenin	0.565	−0.116	0.787	−0.456	1.005	0.672	−0.322	1.112	−0.538	1.043	0.710	−0.395	0.690	−0.669	1.359	0.638	−0.248	0.575	−0.460	0.851
Baicalein	0.261	0.732	6.490	0.161	−0.948	0.123	0.856	1.354	0.694	−1.591	0.060	1.252	3.436	0.785	−2.335	0.151	0.751	1.247	0.628	−1.386
Galangin	0.346	0.296	2.241	0.042	0.046	0.435	0.121	1.926	−0.009	0.054	0.404	0.170	0.665	0.016	0.105	0.431	0.125	1.534	0.001	0.017
Kaempferol	0.478	0.041	1.912	−0.252	0.601	0.419	0.150	1.911	0.025	−0.011	0.442	0.103	1.052	−0.070	0.247	0.407	0.164	0.739	0.055	−0.072
Luteolin	0.186	0.756	4.187	0.400	−1.059	0.134	0.816	1.588	0.669	−1.502	0.098	1.013	3.084	0.698	−1.797	0.130	0.827	1.259	0.675	−1.554
Quercetin	0.268	0.485	3.092	0.218	−0.406	0.180	0.676	2.054	0.565	−1.187	0.143	0.784	1.537	0.600	−1.270	0.174	0.687	1.795	0.577	−1.239
Fisetin	0.254	0.592	4.524	0.254	−0.759	0.184	0.656	1.657	0.556	−1.142	0.240	0.523	2.284	0.378	−0.700	0.226	0.541	1.515	0.460	−0.912
Geraldol	0.267	0.492	3.150	0.220	−0.426	0.366	0.241	1.245	0.147	−0.216	0.262	0.472	2.269	0.329	−0.585	0.369	0.246	2.100	0.140	−0.249
6-methylflavone	0.337	0.345	3.385	0.068	−0.147	0.417	0.163	2.843	0.029	−0.038	0.441	0.109	2.223	−0.076	0.221	0.396	0.196	2.191	0.079	−0.136
6-chloro-7-methylflavone	0.213	0.660	3.832	0.339	−0.837	0.247	0.538	3.291	0.411	−0.878	0.301	0.407	3.172	0.236	−0.451	0.254	0.523	3.379	0.396	−0.862
Daidzein	0.714	−0.406	0.733	−0.787	1.604	0.742	−0.469	0.749	−0.692	1.372	0.721	−0.420	0.656	−0.695	1.416	0.835	−0.790	2.322	−0.902	2.082

diethyl ether solution of fats, except for pig, sheep and pullet fats, which were prepared as 5% solution. The water presence in the margarine and butter led to the necessity of its elimination from the etheric solutions by using a separation funnel previously of impregnation. The pig, pullet, and sheep fats used as raw material were extracted from the natural membranes by heating to melting point followed by a filtration. The obtained fats were used for the impregnation as 5% diethyl ether solution. The human fat was simply dissolved in the diethyl ether by using a porcelain mortar. The impregnation was performed by ascendant development.

The mobile phases containing different mixtures of methanol and water were optimized for each stationary phase's type in order to obtain a significant increase of migration of the compounds while the elution step was changed. In each case were performed 5 steps at different fraction of methanol between 65% and 85% for RP-18, between 55% and 75% for RP-18 W, pig, sheep, pullet and between 50% and 70% for CN, paraffin, TOA, olive, sunflower, corn, margarine, butter, cod liver, and human fat in 5% increments.

RESULTS AND DISCUSSION

The chromatographic lipophilicity indices expressed by mR_F, mR_M, R_{M0}, $PC1/R_F$, and $PC1/R_M$ are listed in Tables 1 to 4. The obtained results indicate that the employed flavonoids cover a large range of lipophilicity scale, which recommends them to be proper for the lipophilicity ranking of the reverse stationary phases. The correlation coefficient between the R_M values and C was higher than 0.96 in a majority of the cases excepting for 3,6-dihydroxyflavone ($r_{TOA} = 0.95$) and chrysine ($r_{TOA} = 0.95$; $r_{Sheep} = 0.95$). By careful examination of the obtained lipophilicity indices it is relatively easy to observe that the 6-chloro-7-methyl-flavone appears to be the most lipophilic compound closely followed by the 6-methyl-flavone, while the daidzein, fisetin, and luteolin presented the lowest lipophilicity. Furthermore, the chromatographic descriptors allow the comparison of the stationary phases through the 2D graphically patterns corresponding to the considered flavonoids. Figure 1 illustrates the correlation patterns between investigated reverse phases according to the R_{M0} values. It is easy to recognize the strongest lipophilic character of the RP-18 plates followed by the RP-18 W. The chromatographic behavior of the flavonoids on the human fat presents high similarities with those observed on paraffin and RP-18 W plates. The lipophilicity indices values on TOA plates are quite different and this result might be explained by the effect of polar interactions. The hydrogen bonding via nitrogen is dominant in this case. Finally, one should mention the high correlation between the vegetable oils and animal fats (Figs. 1a–c). The profiles indicate the corn oil as the most lipophilic vegetable oil closely followed by the sunflower and olive oil and a high similarity between butter, margarine, and human fat. The cod liver oil is the most lipophilic from

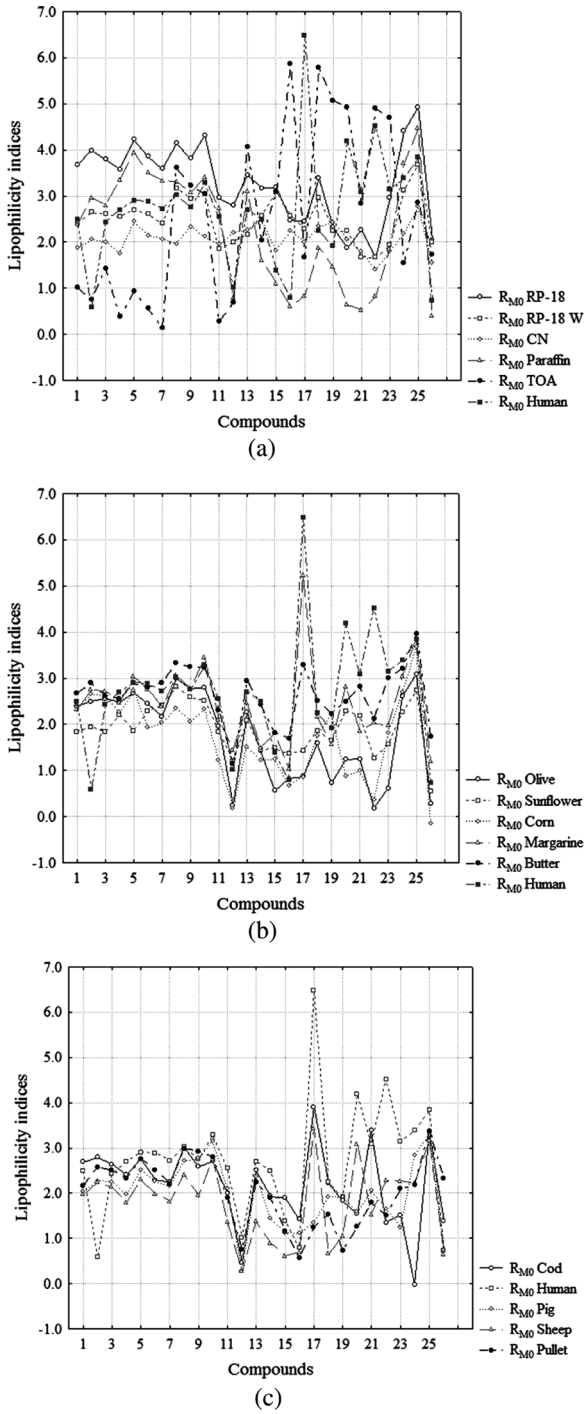


FIG. 1. The correlation patterns of lipophilicity indices corresponding to the investigated reverse stationary phases (RP-18, RP-18 W, CN, paraffin, TOA, human fat, live oil, sunflower oil, corn oil, margarine, butter, cod liver fat, pig fat, sheep fat, and pullet fat).

the animal fats series, while the rest of them are highly correlated.

By applying PCA to the data matrices corresponding to all investigated reverse phases it was found that the first principal component of R_F and R_M accounts in each case more than 90% from the total variation (information), while the first two principal components account even more than 99% in all cases. As a direct consequence, the scores corresponding to the first principal component can be used as a new lipophilcity scale. The reliability of the scores values as lipophilic indices are shown by their significant correlation with the classical R_{M0} values and other lipophilicity indices. In addition, the ''lipophilicity chart'' described by the first two principal components has the effect of separating compounds from each other mostly effectively from the congeneric (similarity) point of view (22–27). This approach can be extended also to the matrix of R_{M0} values corresponding to all investigated phases. The lipophilicity charts obtained by PC1-PC2 score plot corresponding to different lipophilicity indices estimated on all investigated reverse stationary phases are shown in Figs. 2a–e. It appears clearly that the reverse phases studied in this paper form practically linear clusters. Once more, the human fat results are highly correlated with those obtained on margarine and butter impregnated plates. Using as lipophilicity indices the $PC1/R_F$ or $PC1/R_M$ a high analytical level linearization of the results can be observed. Moreover, these aspects sustain the wide

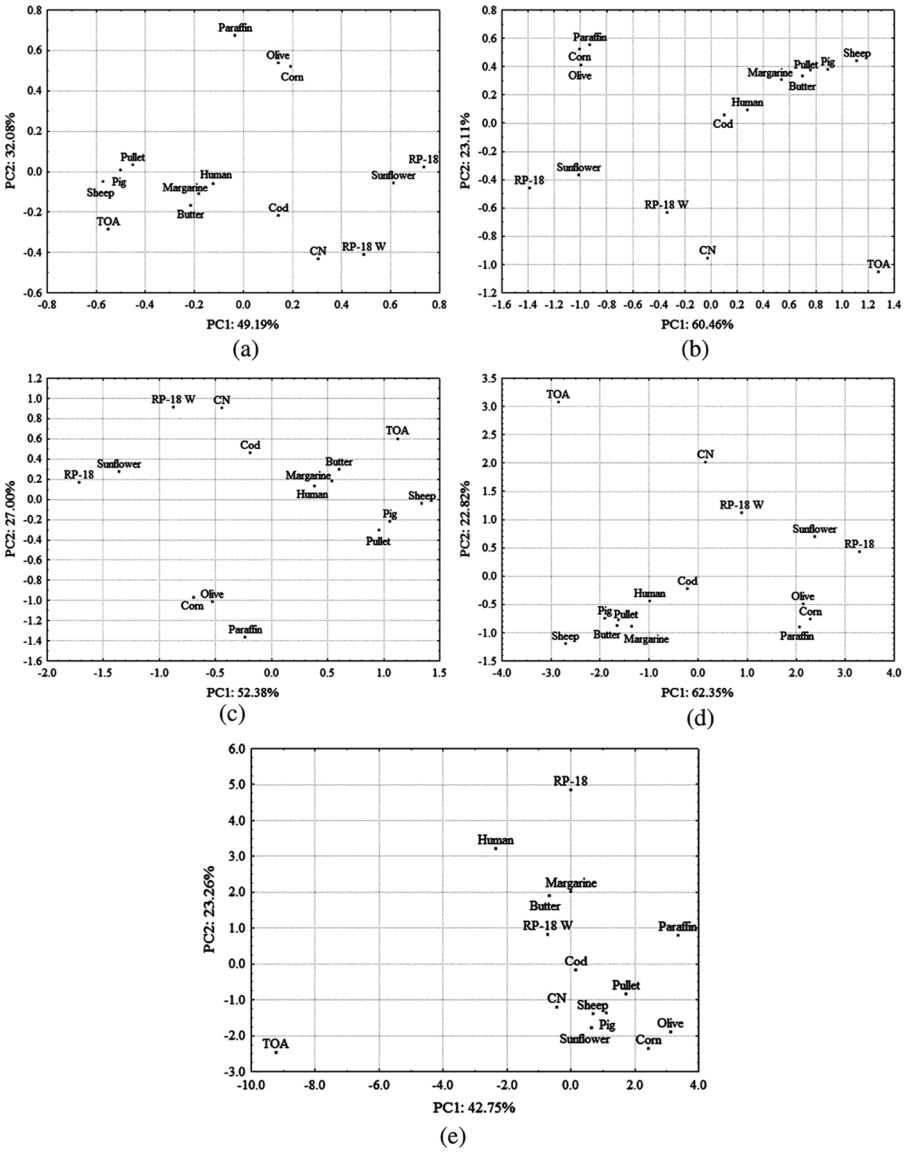
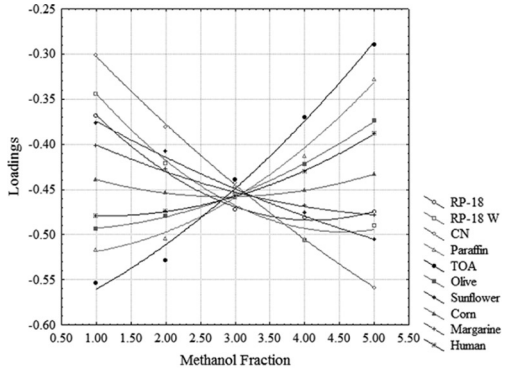
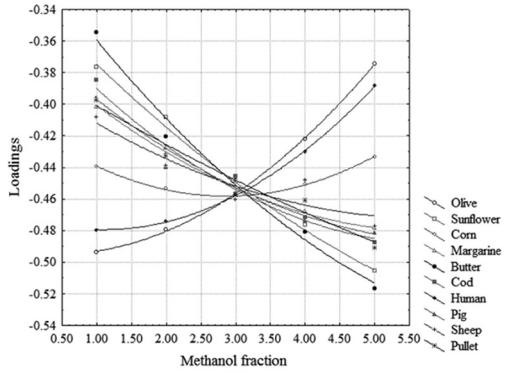


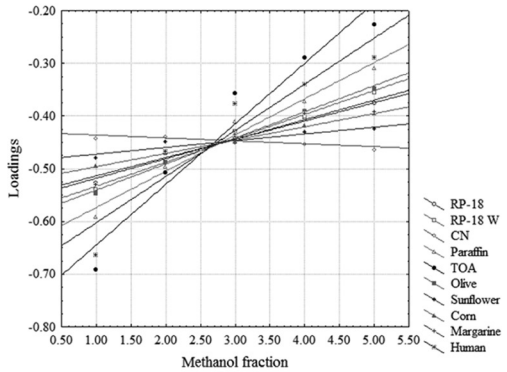
FIG. 2. The lipophilicity charts obtained by PC1-PC2 score plot corresponding to different lipophilicity indices estimated on all investigated reverse stationary phases: mR_F (a); $PC1/R_F$ (b); mR_M (c); $PC1/R_M$ (d); R_{M0} (e).



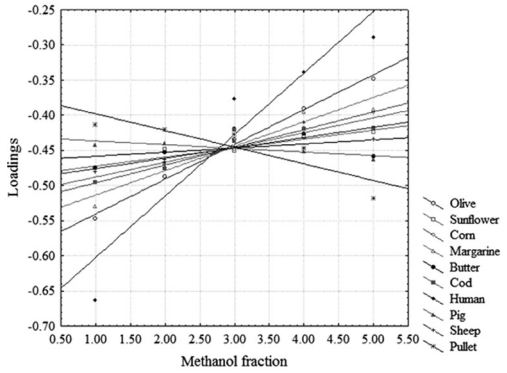
(a)



(b)



(c)



(d)

FIG. 3. Profiles of loadings corresponding to the first principal component obtained by applying PCA to R_F values (a, b) and R_M values (c, d).

TABLE 5
The correlation concerning results obtained on human fat-impregnated plates vs. the chemically bonded plates and synthetic oils-impregnated plates

		Human				
Reverse phase	Lipophilicity index	mR_F	mR_M	R_{M0}	PC1/R_F	PC1/R_M
RP-18	mR_F	0.34	−0.27	−0.08	−0.34	0.26
	mR_M	−0.38	0.31	0.10	0.38	−0.30
	R_{M0}	−0.30	0.22	−0.03	0.31	−0.21
	PC1/R_F	−0.34	0.27	0.08	0.34	−0.26
	PC1/R_M	0.38	−0.31	−0.10	−0.38	0.30
RP-18 W	mR_F	**0.73**	−0.66	−0.36	**−0.74**	0.66
	mR_M	**−0.71**	0.64	0.33	**0.71**	−0.64
	R_{M0}	−0.32	0.28	−0.02	0.33	−0.26
	PC1/R_F	**−0.73**	0.66	0.36	**0.74**	−0.65
	PC1/R_M	**0.70**	−0.64	−0.32	**−0.71**	0.63
CN	mR_F	0.64	−0.59	−0.22	−0.64	0.57
	mR_M	−0.61	0.56	0.20	0.61	−0.54
	R_{M0}	−0.18	0.14	−0.03	0.18	−0.12
	PC1/R_F	−0.64	0.59	0.22	0.64	−0.57
	PC1/R_M	0.60	−0.56	−0.20	−0.61	0.54
Paraffin	mR_F	0.69	−0.63	−0.37	**−0.70**	0.63
	mR_M	−0.68	0.63	0.37	0.69	−0.63
	R_{M0}	−0.32	0.24	0.10	0.33	−0.24
	PC1/R_F	−0.68	0.62	0.36	0.69	−0.62
	PC1/R_M	0.66	−0.61	−0.35	−0.67	0.61
TOA	mR_F	0.55	−0.57	−0.31	−0.55	0.55
	mR_M	−0.52	0.53	0.27	0.51	−0.51
	R_{M0}	−0.28	0.29	0.07	0.28	−0.28
	PC1/R_F	−0.54	0.55	0.29	0.53	−0.54
	PC1/R_M	0.48	−0.49	−0.24	−0.48	0.48

capacity of the PCA to create an acceptable hierarchy. The graphical arrangements place the human fats as intermediary between cod liver and pullet, which may sustain the supposition that the human diet has to be concentrated on white meat. Much more, the PCA might be used for investigating the retention mechanism involved in the development process by examination of the profile of loadings/eigenvectors corresponding to the first principal component. The quadratic profile of loadings presented in Figs. 3a–b (R_F values) and linear profiles Figs. 3c–d (R_M values) illustrate once again the differences and similarity between the investigated reversed-phases; a high regular (linear) retention behavior (Fig. 3a) can be easily observed in the case of CN and sunflower oil-impregnated plates by comparing with all others.

All the statements above are well supported by the correlation matrices of the chromatographic lipophilicity

TABLE 6
The correlation concerning results obtained on human fat-impregnated plates vs. the vegetable oils, margarine and butter-impregnated plates

Reverse Phase	Lipophilicity index	Human mR_F	Human mR_M	Human R_{M0}	Human PC1/R_F	Human PC1/R_M
Olive	mR_F	0.70	−0.63	−0.30	−0.72	0.62
	mR_M	−0.71	0.64	0.31	0.72	−0.64
	R_{M0}	−0.43	0.35	0.12	0.44	−0.35
	PC1/R_F	−0.70	0.62	0.30	0.71	−0.62
	PC1/R_{M0}	0.70	−0.63	−0.30	−0.71	0.63
Sunflower	mR_F	0.55	−0.45	−0.10	−0.56	0.44
	mR_M	−0.57	0.48	0.13	0.58	−0.47
	R_{M0}	−0.65	0.59	0.31	0.67	−0.59
	PC1/R_F	−0.54	0.45	0.09	0.56	−0.43
	PC1/R_M	0.57	−0.49	−0.13	−0.58	0.47
Corn	mR_F	0.70	−0.64	−0.35	−0.71	0.63
	mR_M	−0.70	0.64	0.34	0.70	−0.64
	R_{M0}	−0.44	0.35	0.10	0.45	−0.35
	PC1/R_F	−0.70	0.64	0.35	0.71	−0.63
	PC1/R_M	0.69	−0.64	−0.34	−0.70	0.63
Margarine	mR_F	**0.97**	**−0.98**	−0.79	**−0.96**	**0.98**
	mR_M	**−0.93**	**0.97**	**0.82**	**0.92**	**−0.97**
	R_{M0}	−0.62	0.69	0.74	0.60	−0.69
	PC1/R_F	**−0.97**	**0.98**	0.79	**0.96**	**−0.98**
	PC1/R_M	**0.93**	**−0.97**	**−0.82**	**−0.92**	**0.97**
Butter	mR_F	**0.97**	**−0.98**	−0.78	**−0.96**	**0.98**
	mR_M	**−0.95**	**0.98**	**0.80**	**0.94**	**−0.97**
	R_{M0}	−0.76	0.74	0.58	0.75	−0.73
	PC1/R_F	**−0.97**	**0.98**	0.78	**0.96**	**−0.98**
	PC1/R_M	**0.95**	**−0.98**	**−0.80**	**−0.94**	**0.97**

TABLE 7
The of correlation concerning results obtained on human fat-impregnated plates vs. the vegetable oils, margarine and butter-impregnated plates

Reverse phase	Lipophilicity index	Human mR_F	Human mR_M	Human R_{M0}	Human PC1/R_F	Human PC1/R_M
Cod	mR_F	**0.93**	**−0.91**	−0.67	**−0.93**	**0.91**
	mR_M	**−0.92**	**0.92**	0.67	**0.92**	**−0.92**
	R_{M0}	−0.46	0.46	0.36	0.44	−0.44
	PC1/R_F	**−0.93**	**0.91**	0.66	**0.93**	**−0.91**
	PC1/R_M	**0.92**	**−0.92**	−0.67	**−0.92**	**0.92**
Pig	mR_F	**0.91**	**−0.95**	**−0.82**	**−0.90**	**0.95**
	mR_M	**−0.89**	**0.94**	**0.83**	**0.88**	**−0.94**
	R_{M0}	−0.60	0.53	0.30	0.61	−0.54
	PC1/R_F	−0.91	**0.95**	**0.82**	**0.90**	**−0.95**
	PC1/R_M	0.89	**−0.94**	**−0.83**	**−0.88**	**0.94**
Sheep	mR_F	0.90	**−0.93**	−0.79	**−0.89**	**0.93**
	mR_M	−0.84	**0.90**	0.82	**0.83**	**−0.90**
	R_{M0}	−0.75	**0.80**	0.77	0.74	**−0.80**
	PC1/R_F	**−0.90**	**0.93**	0.79	**0.88**	**−0.93**
	PC1/R_M	**0.85**	**−0.90**	**−0.82**	**−0.83**	**0.90**
Pullet	mR_F	**0.93**	**−0.95**	**−0.80**	**−0.92**	**0.95**
	mR_M	**−0.92**	**0.94**	**0.80**	**0.91**	**−0.94**
	R_{M0}	−0.42	0.36	0.13	0.43	−0.36
	PC1/R_F	**−0.93**	**0.95**	**0.80**	**0.92**	**−0.95**
	PC1/R_M	**0.92**	**−0.94**	**−0.80**	**−0.91**	**0.94**

indices obtained on the investigated stationary phases (Tables 5 to 7). The best correlations ($r = 0.98$) were found between the $mR_{M,\ Human}$ with $mR_{F,\ Margarine}$, PC1/$R_{F,\ Margarine}$, $mR_{F,\ Butter}$, $mR_{M,\ Butter}$, PC1/$R_{F,\ Butter}$, PC1/$R_{M,\ Butter}$, and PC1/$R_{M,\ Human}$ with $mR_{F,\ Margarine}$, PC1/$R_{F,\ Margarine}$, $mR_{F,\ Butter}$ and, respectively PC1/$R_{F,\ Butter}$. High correlation coefficients were found as well between the human fat lipophilicity indices with all the animal and vegetable fats. The lowest correlations were found for the RP-18, followed by TOA. The RP-18 W and paraffin oil plates provided values closer by those obtained on the rest of the plates ($r > 0.70$).

CONCLUSIONS

The chromatographic lipophilicity indices obtained for a series of related flavonoids are a valuable source of information which may be used for an objective characterization of TLC plates impregnated with fats. The human fat-impregnated plates provided lipophilicity values closely associated with those obtained for margarine and butter. Moreover, the human fat lipophilic character seems to be placed between pullet and cod fats. Even if there are some unknown aspects which define the chromatographic mechanism, especially because of the variable chemical composition of the fats, the PCA offers useful information concerning classification and ranking aspects by the characteristic lipophilicity charts and by graphically representation of the loadings. Once more the PCA shows its high capacity within the analytical characterization of different chemical processes including the chromatographic mechanism.

ACKNOWLEDGEMENTS

The financial support of the Ministry of Education, Research and Innovation of Romania (ID 560/2007–2010) is gratefully acknowledged.

REFERENCES

1. http://goldbook.iupac.org/LT06965.html
2. Kaliszan, R. (2007) QSRR: Quantitative structure-(chromatographic) retention relationships. *Chemical Reviews*, 107: 3212–3246.
3. Testa, B.; Crivori, P.; Reist, M.; Carrupt, P.A. (2000) The influence of lipophilicity on the pharmacokinetic behavior of drugs: Concepts and exemples. *Perspectives in Drug Discovery and Design*, 1: 179–211.
4. Kaliszan, R. (1997) *Structure and Retention in Chromatography: A Chemometric Approach*; Harwood Academic Publishers: Amsterdam.
5. Hansch, H.; Leo, A. (1995) *Exploring QSAR. Fundamentals and Applications in Chemistry and Biology*; ACS: Washington, DC.
6. Huber, J.F.K.; Meijers, C.A.H.; Hulsman, J.A.R.J. (1972) Prediction of coefficients in liquid-liquid systems and its experimental verification for steroids by statistic and chromatographic measurements. *Analytical Chemistry*, 44: 111–116.
7. Kaune, A.; Knorrenschild, M.; Kettrup, A. (1995) Predicting 1-octanol-water partition coefficients by high performance liquid chromatography gradient elution. *Fresenius Journal of Analytical Chemistry*, 352: 303–312.
8. Djaković-Sekulić, T.L.; Sârbu, C.; Perišić-Janjić, N.U. (2005) A comparative study of the lipophilicity of benzimidazole and benztriazole derivatives by RPTLC. *Journal of Planar Chromatography*, 18: 432–436.
9. Perišić-Janjić, N.U.; Djaković-Sekulić, T.L. (2006) Study of the characteristics and separation properties of unconventional TLC supports. Part I. *Journal of Planar Chromatography*, 19: 438–442.
10. Pyka, A.; Babuśka, M.; Śliwiok, J. (2006) Use of liquid chromatography and theoretical computational methods to compare the lipophilicity of selected cortisone derivatives. *Journal of Planar Chromatography*, 19: 432–437.
11. Djaković-Sekulić, T.L.; Perišić-Janjić, N.U. (2007) Study of the characteristics and separating power of unconventional TLC supports. II. Principal-components analysis. *Journal of Planar Chromatography*, 20: 7–11.
12. Csermely, T.; Petroianu, G.; Kuca, K.; Fûrész, J.; Darvas, F.; Gulyás, Z.; Laufer, R.; Kalász, H. (2007) TLC of quaternary pyridinium aldoximes, antidotes of organophosphorus esterase inhibitors. *Journal of Planar Chromatography*, 20: 39–42.
13. Kresta, J.; Kastner, P.; Klimeš, J.; Klimešová, V. (2005) Reversed-phase thin-layer chromatographic determination of the lipophilicity of potential antituberculotic compounds. *Journal of Planar Chromatography*, 18: 450–454.
14. Mrkvičková, Z.; Kovaříková, P.; Klimeš, J.; Doležal, M. (2006) Determination of the lipophilicity of potential antituberculotic compounds by RP-TLC. *Journal of Planar Chromatography*, 19: 422–426.
15. Pyka, A. (2004) New method of calculation of the partition coefficients of selected alkoxyphenols investigated by RPTLC. *Journal of Planar Chromatography*, 17: 58–60.
16. http://www.sunflowernsa.com/uploads/NuSun_FactSheet_vol_1.3.pdf
17. http://www.corn.org/CornOil.pdf
18. Lascu, D. (2008) *Food Encyclopedia*; All: Bucharest.
19. Kowalska, T.; Kaczmarski, K.; Prus, W. (2003) In: *Handbook of Thin Layer Chromatography*, Sherma, J.; Fried, B., eds.; Marcel Dekker: New York, 47–80.
20. Sangster, J. (1997) *Octanol-Water Partition Coefficients: Fundamentals and Physical Chemistry*; Wiley: West Sussex.
21. Bate-Smith, E.C.; Westall, R.G. (1950) Chromatographic behaviour and chemical structure I. Some naturally occuring phenolic substances. *Biochimica et Biophysica Acta*, 4: 427–440.
22. Casoni, D.; Kot-Wasik, A.; Namieśnik, J.; Sârbu, C. (2009) Lipophilicity data for some preservatives estimated by reversed-phase liquid chromatography and different computation methods. *Journal of Chromatography A*, 1216: 2456–2465.
23. Sârbu, C.; Tudor, S. (1998) Determination of lipophilicity of some non-steroidal anti-inflammatory agents and their relationships by using principal component analysis based on thin-layer chromatographic retention data. *Journal of Chromatography A*, 822: 263–269.
24. Sârbu, C.; Casoni, D.; Darabantu, M.; Maiereanu, C. (2004) Quantitative structure—retention and retention—activity relationships of some 1,3-oxazolidine systems by RP-HPTLC and PCA. *Journal of Pharmaceutical and Biomedical Analysis*, 35: 213–219.
25. Sârbu, C.; Djaković-Sekulić, T.L.; Perišić-Janjić, N.U. (2002) Evaluation of lipophilicity of some benzimidazole and benztriazole derivatives by RP HPTLC and PCA. *Journal of Pharmaceutical and Biomedical Analysis*, 30: 739–745.
26. Briciu, R.D.; Kot-Wasik, A.; Namiesnik, J.; Sârbu, C. (2009) A comparative study of the molecular lipophilicity indices of vitamins A and E, and of some precursors of vitamin A, estimated by HPLC and by different computation methods. *Acta Chromatographica*, 21: 237–250.
27. Briciu, R.D.; Kot-Wasik, A.; Namiesnik, J.; Sârbu, C. (2009) The lipophilicity indices of flavonoids estimated by reverse phase liquid chromatography using different computation methods. *Journal of Separation Science*, 32: 2066–2074.
28. http://www.statistica.com