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COMPARATIVE STUDY OF DIFFERENT OILS AND FATS IMPREGNATED THIN-LAYER CHROMATOGRAPHIC LAYERS FOR THE AMINO ACIDS LIPOPHILICITY ESTIMATION

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ABSTRACT. The chromatographic behavior of a series of amino acids compounds was investigated on silica gel chromatographic plates impregnated with various oils (paraffin, olive, sunflower and corn) and different animal fats (pig, pullet, sheep and bear) using mixture of methanol-phosphate buffer in different proportions as mobile phase. The relevance of the obtained results was evaluated by a critical comparison of the lipophilicity parameters with different theoretical lipophilicity and solubility indices. Also some correlation matrices and diagrams were developed for a comparative evaluation of the studied impregnated stationary phases. The results indicated that the oils and some animal fats (pullet and bear) impregnated silica gel plates can be a good alternative in the field of chromatographic lipophilicity estimation of amino acids. In addition, the PCA methodology proved again to offer a realistic characterization of the impregnated plates, both from the retention mechanism and lipophilicity point of view.

***Keyords**: TLC, lipophilicity, amino acids, impregnated TLC plates*

INTRODUCTION

A problem that continues to evade researchers is a complete understanding of how proteins fold into their native state. The importance of this problem lies in the interactions of the individual amino acids that make up the tertiary structure. From the four types of involved interactions (hydrophobic/ lipophilic, electrostatic, hydrogen bonding, and van der Waals), the hydrophobic/ lipophilic ones are believed to be the most significant [1] giving considerable insights into how a protein is going to fold. A better understanding of these interactions can be provided by the lipophilicity concept that has been examined for many decades in absorption, permeability, toxicity and *in vivo* distribution of organic compounds [2]. Over the years, a vast amount of work has been done in measuring amino acids lipophilicity in order to find a universal amino acid lipophilicity scale that would be ideal in examining the interactions of

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transmembrane peptide segments with lipid bilayers (the natural environment of such peptides) [3]. The lipophilicity, defined as the tendency of a compound to partition between non-polar and aqueous environments, is most commonly measured directly using the shake-flask technique (when lipophilicity is expressed by log P_{ow} or log k_{ow} values) or indirectly using reversed-phase liquid chromatography (when lipophilicity is expressed by log k_w or R_{M0} values). Because of some advantages, nowadays the shake-flask technique has been successfully replaced by chromatographic methods such as high performance liquid chromatography RP-HPLC [4-7] and thin layer chromatography (RP-HPTLC) [8-10]. Concerning the experimental estimation of lipophilicity, the chromatographic procedures offer large possibilities, the combinations between both stationary and mobile phases being practically unlimited. Furthermore, the possibility of impregnation of the HPTLC plates with a series of oils more or less lipophilic may suggest the retention mechanism and may define them in the context of the strength of lipophilicity character. In addition, the chemical composition of vegetable oils (triglicerides, free fatty acids, lipophilic vitamins) and of animal fats (high concentration of saturated fatty acids and cholesterol) may enable their use as new realistic models for the mimesis of biological membranes. Over the years, the paraffin oil [11], silicon oil [12, 13] vegetable oils and different animal fats [14] were successfully used for the impregnation of TLC-plates in order to change the stationary phase characteristics and improve the chromatographic performance. Considering that the lipophilicity experiments are performed mainly to evidence in the *in vivo* behavior of active compounds, it may be appreciated that actually is still a need for continuously improvement of the stationary phases in order to offer a realistic alternative to the investigations of the biological membranes properties.

In the above considerations, the purpose of this work was to investigate the chromatographic behavior of the amino acids on different oils and fats impregnated TLC silica gel plates and evaluate their lipophilicity by using also different computed log P values.

THEORETICAL BACKGROUND

The most popular lipophilicity indices measured by RP-HPTLC are derived from the retention factor (R_F) according to Bate-Smith and Westall [15] equation:

$$R_M = \log (1/R_F - 1) \quad (1)$$

The direct influence of the organic modifier concentration from the mobile phase over the R_M values is described by the linear relationship expressed by the Soczewiński-Wachtmeister equation:

$$R_M = R_{M0} + bC \quad (2)$$

were R_{M0} is the extrapolated value to a zero fraction of organic component in the mobile phase composition, b is the regression slope frequently associated with the specific hydrophobic surface area of the stationary phase and C represents the volume fraction of the organic solvent in the mobile phase composition. Many studies suggested that the biological activity cannot be associated only with R_{M0} values, especially when polar interactions may take place. The specific hydrophobic surface area of the compounds plays an important role, a confirmed fact by the R_{M0} and b correlation [16]. Due to the advanced computerized procedures of multivariate data analysis, more recently, the Principal Component Analysis (PCA) has been successfully applied to develop new lipophilicity indices based on the R_F and R_M values [17,18]. The methodology based on PCA is not only more robust to different errors but it is also more informative. Usually, scatterplot of the first principal components produces charts in which the coordinates of the analytes reproduce the most variance of the input chromatographic data [19]. In addition, the first principal components can offer more efficient alternatives for characterization and ranking of investigated compounds and stationary phases including new insights into the chromatographic behavior of the compounds and the retention mechanism.

RESULTS AND DISCUSSION

For the studied amino acids, the use of different oils and animal fats impregnated silica gel plates revealed a linear dependence of retention parameters (R_M) with methanol fraction in the mobile phase, the regression determination coefficient being higher than 0.98 in all cases. These chromatographic regularities are supported by the profiles of retention parameters representation (Figure 1a and Figure 1b) that also illustrate high similarities in chromatographic behavior of compounds between oils impregnated stationary phases and also between animal fats impregnated stationary phases. This representation proves to be a very good way also for emphasizing the some specific interactions with stationary phases as it is highlighted for phenylalanine on RP-18W and oils impregnated plates (Figure 1a).

All the computed lipophilicity indices (Table 1 and Table 2) and the experimental ones (Table 3 and respectively Table 4) expressed by mean of retention parameters (mR_M), those obtained by extrapolation (R_{M0}), and respectively those obtained by applying PCA on the R_M values ($PC1/R_M$) show the histidine, arginine and respectively lysine as the most lipophilic compounds exception in case of RP-18W stationary phase were phenylalanine followed by tyrosine and respectively leucine seem to have the highest lipophilicity. The particular distinct behavior of lysine, arginine and histidine in case of the impregnated TLC stationary phases (Figure 2) might be attributed to possible specific (hydrogen bond or N-N pair) interactions with some of the principal constituents (lipids, triglycerides, fatty acids, lipoproteins) of the used fats and oils. These interactions seem to change the retention characteristics of the stationary phases and influence the behavior of the compounds containing

multiple amino groups. These considerations are very well supported by the new “lipophilicity charts” provided by the scores corresponding to R_M values onto the planes described by the first two principal components (PC1 and respectively PC2 obtained by applying principal component analysis to R_M values) (Figure 3). The applied methodology classified the specified compounds as outliers of the group in all cases.

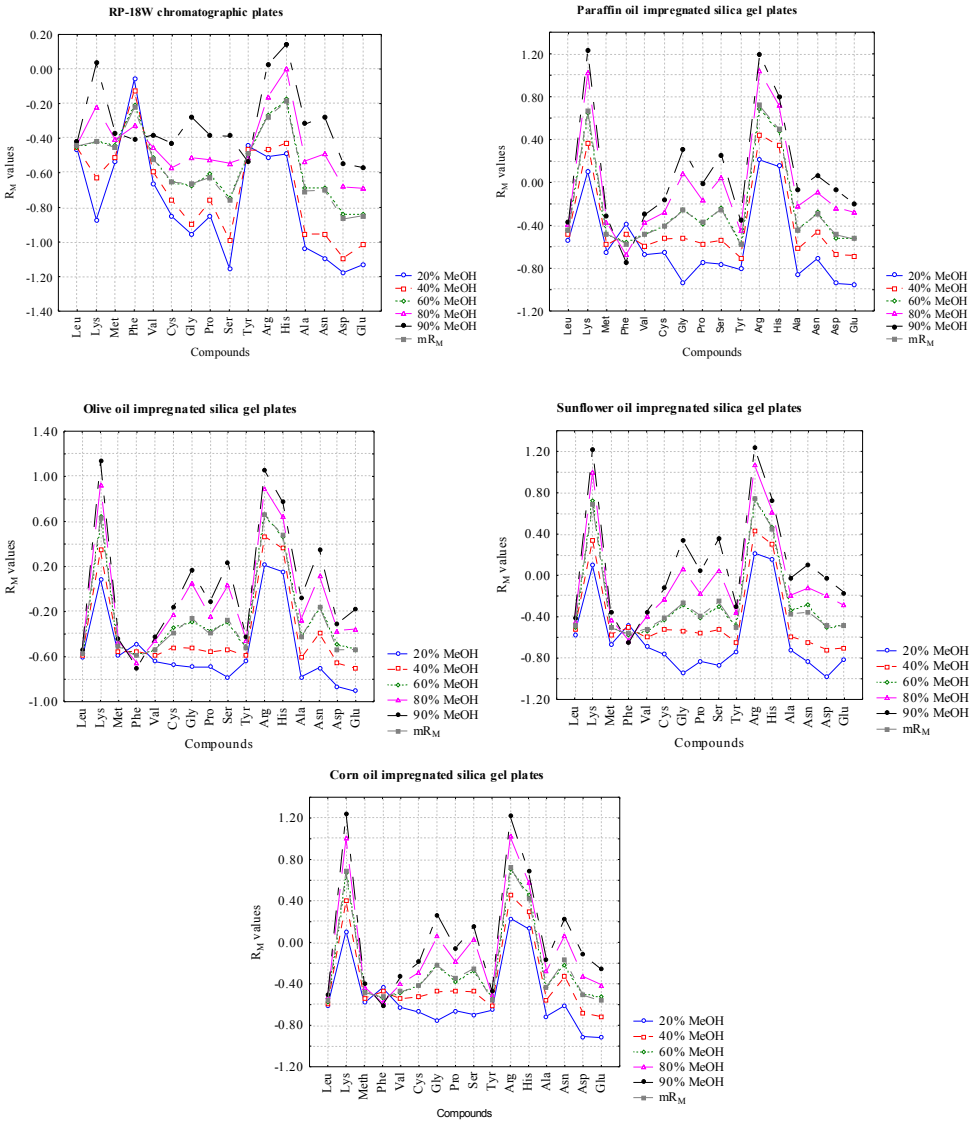


Figure 1a. Profiles of R_M values for all fraction of methanol on the investigated RP and oils impregnated stationary phases.

Table 1. The experimental log P_{ow} and computed lipophilicity descriptors of studied amino acids

No.	Abbr. Cpd.	Log P_{ow}	CLogP	ALOGP	MLOGP	ALOGPs	AC logP	AB/logP	miLogP	XLOGP2	XLOGP3
1	Leu	-1.52	-1.667	0.631	-1.677	-1.82	0.20	-1.77	-1.38	-1.39	-1.52
2	Lys	-3.05	-3.424	-0.680	-2.485	-3.76	-1.16	-2.00	-3.18	-2.95	-3.05
3	Met	-1.85	-1.730	-0.273	-2.055	-1.85	-0.43	-2.00	-2.24	-1.85	-1.87
4	Phe	-1.38	-1.556	0.955	-0.968	-1.35	0.21	-1.39	-1.23	-1.38	-1.52
5	Val	-2.26	-2.286	0.242	-2.055	-2.29	-2.26	-2.00	-1.91	-2.17	-2.26
6	Cys	-2.49	-2.347	-0.517	-2.918	-2.57	-0.94	-2.00	-2.71	-2.57	-2.49
7	Gly	-3.21	-3.210	-0.978	-3.437	-3.34	-1.47	-2.00	-2.55	-3.35	-3.21
8	Pro	-2.54	-2.413	-0.057	-0.232	-2.71	-0.35	-2.00	-1.7	-0.18	-2.50
9	Ser	-3.07	-2.811	-1.489	-3.726	-3.42	-2.02	-2.00	-3.67	-3.96	-3.07
10	Tyr	-2.26	-2.223	0.688	-1.508	-2.39	-0.09	-2.00	-1.71	-1.78	-2.26
11	Arg	-4.20	-3.517	-1.107	-2.934	-3.49	-2.41	-2.00	-3.63	-2.97	-4.20
12	His	-3.32	-4.367	-1.015	-3.057	-2.67	-1.64	-2.00	-3.00	-3.11	-3.56
13	Ala	-2.85	-3.124	-0.601	-2.918	-3.05	-1.06	-2.00	-2.69	-2.82	-2.96
14	Asn	-3.82	-3.544	-1.847	-3.762	-3.36	-2.49	-2.00	-2.81	-4.43	-3.41
15	Asp	-3.89	-2.411	-1.245	-3.356	-3.52	-1.95	-2.00	-3.52	-3.71	-2.76
16	Glu	-3.69	-2.694	-0.924	-2.946	-3.54	-1.49	-2.00	-3.25	-3.35	-3.69

Table 2. The solubility values for the studied amino acids

No.	Abbr. Cpd.	S_{exp} (mg/mL)	ALOGpS	AC logS
1	Leu	21.5	-0.27	-1.11
2	Lys	1000.0	-0.14	-0.79
3	Met	56.6	-0.80	-1.05
4	Phe	26.9	-1.60	-1.54
5	Val	58.5	0.26	-0.84
6	Cys	277.0	-0.72	-1.06
7	Gly	249.0	0.87	-0.03
8	Pro	162.0	0.50	-0.71
9	Ser	425.0	0.66	0.10
10	Tyr	0.5	-1.37	-1.25
11	Arg	182.0	-1.88	-0.16
12	His	45.6	-0.34	-0.38
13	Ala	164.0	0.70	-0.41
14	Asn	29.4	0.10	-0.23
15	Asp	5.4	0.03	-0.15
16	Glu	8.6	-0.26	-0.42

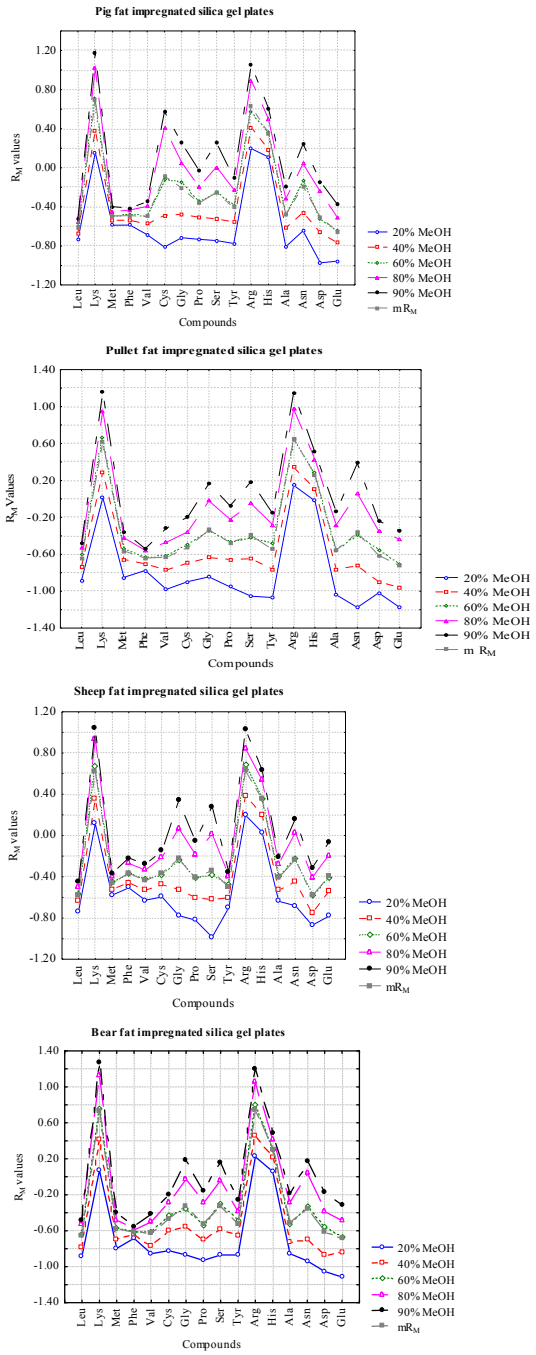


Figure 1b. Profiles of R_M values for all fraction of methanol on the investigated animal fats impregnated stationary phases.

Table 3. The lipophilicity indices of amino acids obtained on RP-18W and different oils impregnated TLC plates

No.	Abbr. Cpd.	RP-18W				Paraffin				Olive				Sunflower				Corn			
		mR_M	R_{M0}	b	PC1/ R_M	mR_M	R_{M0}	b	PC1/ R_M	mR_M	R_{M0}	b	PC1/ R_M	mR_M	R_{M0}	b	PC1/ R_M	mR_M	R_{M0}	b	PC1/ R_M
1	Leu	-0.445	-0.472	0.001	-0.341	-0.444	-0.581	0.002	0.534	-0.573	-0.636	0.001	0.790	-0.486	-0.617	0.002	0.627	-0.559	-0.651	0.002	0.791
2	Lys	-0.420	-0.930	0.012	-0.161	0.671	-0.256	0.016	-2.059	0.626	-0.229	0.015	-1.991	0.679	-0.259	0.016	-2.076	0.684	-0.234	0.016	-2.096
3	Met	-0.453	-0.554	0.002	-0.289	-0.483	-0.756	0.005	0.594	-0.512	-0.636	0.002	0.644	-0.506	-0.755	0.004	0.650	-0.483	-0.641	0.003	0.612
4	Phe	-0.226	-0.017	-0.005	-0.913	-0.569	-0.284	-0.005	0.881	-0.596	-0.427	-0.003	0.884	-0.567	-0.414	-0.003	0.852	-0.528	-0.386	-0.003	0.768
5	Val	-0.520	-0.681	0.004	-0.119	-0.486	-0.795	0.005	0.596	-0.531	-0.714	0.003	0.675	-0.515	-0.800	0.005	0.665	-0.477	-0.712	0.004	0.584
6	Cys	-0.654	-0.896	0.006	0.199	-0.408	-0.796	0.007	0.409	-0.384	-0.811	0.007	0.307	-0.417	-0.922	0.009	0.412	-0.420	-0.804	0.007	0.430
7	Gly	-0.663	-1.071	0.010	0.296	-0.262	-1.257	0.017	-0.015	-0.257	-0.994	0.013	-0.029	-0.271	-1.285	0.018	0.006	-0.225	-1.050	0.014	-0.082
8	Pro	-0.626	-0.897	0.007	0.155	-0.377	-0.973	0.010	0.305	-0.397	-0.870	0.008	0.327	-0.388	-1.063	0.012	0.321	-0.348	-0.823	0.008	0.255
9	Ser	-0.761	-1.214	0.011	0.527	-0.249	-1.088	0.015	-0.019	-0.270	-1.102	0.014	-0.020	-0.256	-1.220	0.017	-0.019	-0.251	-0.961	0.012	-0.004
10	Tyr	-0.490	-0.439	-0.001	-0.284	-0.577	-0.952	0.007	0.785	-0.527	-0.707	0.003	0.667	-0.506	-0.876	0.006	0.630	-0.558	-0.713	0.003	0.778
11	Arg	-0.278	-0.598	0.008	-0.551	0.715	-0.106	0.014	-2.137	0.658	-0.020	0.012	-2.029	0.736	-0.122	0.015	-2.190	0.726	-0.080	0.014	-2.164
12	His	-0.190	-0.586	0.009	-0.698	0.499	-0.046	0.009	-1.613	0.476	-0.006	0.008	-1.592	0.452	-0.016	0.008	-1.497	0.426	-0.022	0.008	-1.437
13	Ala	-0.705	-1.138	0.010	0.398	-0.441	-1.078	0.011	0.441	-0.432	-0.988	0.010	0.387	-0.372	-0.943	0.010	0.301	-0.427	-0.864	0.008	0.435
14	Asn	-0.700	-1.187	0.012	0.413	-0.295	-0.912	0.011	0.123	-0.154	-0.990	0.014	-0.274	-0.355	-1.130	0.013	0.230	-0.172	-0.836	0.012	-0.169
15	Asp	-0.867	-1.255	0.009	0.716	-0.489	-1.188	0.012	0.535	-0.539	-0.997	0.008	0.642	-0.488	-1.264	0.013	0.523	-0.509	-1.122	0.011	0.579
16	Glu	-0.849	-1.185	0.008	0.653	-0.530	-1.144	0.011	0.640	-0.536	-1.114	0.010	0.613	-0.489	-1.029	0.009	0.565	-0.564	-1.086	0.009	0.720

Table 4. The lipophilicity indices of amino acids obtained on different animal fats impregnated TLC plates

No.	Abbr. Cpd.	Pig				Pullet				Sheep				Bear			
		mR_M	R_{M0}	b	$PC1/R_M$	mR_M	R_{M0}	b	$PC1/R_M$	mR_M	R_{M0}	b	$PC1/R_M$	mR_M	R_{M0}	b	$PC1/R_M$
1	Leu	-0.615	-0.794	0.003	0.925	-0.644	-0.976	0.006	0.702	-0.574	-0.805	0.004	0.797	-0.663	-1.003	0.006	0.800
2	Lys	0.688	-0.173	0.015	-2.059	0.618	-0.331	0.016	-2.164	0.624	-0.166	0.014	-1.933	0.730	-0.283	0.018	-2.374
3	Met	-0.492	-0.642	0.003	0.657	-0.565	-0.961	0.007	0.521	-0.462	-0.640	0.003	0.555	-0.591	-0.913	0.006	0.642
4	Phe	-0.489	-0.635	0.003	0.652	-0.642	-0.843	0.004	0.711	-0.364	-0.609	0.004	0.329	-0.618	-0.719	0.002	0.728
5	Val	-0.499	-0.775	0.005	0.653	-0.632	-1.150	0.009	0.657	-0.438	-0.725	0.005	0.488	-0.631	-0.998	0.006	0.726
6	Cys	-0.085	-1.267	0.020	-0.402	-0.528	-1.083	0.010	0.422	-0.363	-0.735	0.006	0.309	-0.466	-0.970	0.009	0.343
7	Gly	-0.206	-0.998	0.014	-0.073	-0.337	-1.180	0.015	-0.030	-0.225	-1.122	0.016	-0.064	-0.325	-1.167	0.015	-0.009
8	Pro	-0.362	-0.912	0.010	0.308	-0.474	-1.179	0.012	0.288	-0.413	-1.041	0.011	0.388	-0.522	-1.148	0.011	0.452
9	Ser	-0.251	-1.056	0.014	0.023	-0.397	-1.382	0.017	0.092	-0.338	-1.351	0.018	0.172	-0.326	-1.161	0.014	-0.006
10	Tyr	-0.410	-0.949	0.009	0.416	-0.548	-1.295	0.013	0.451	-0.505	-0.798	0.005	0.635	-0.531	-1.015	0.008	0.489
11	Arg	0.628	-0.073	0.012	-1.901	0.653	-0.191	0.015	-2.235	0.629	-0.052	0.012	-1.929	0.749	-0.077	0.014	-2.392
12	His	0.355	-0.064	0.007	-1.256	0.261	-0.183	0.008	-1.323	0.353	-0.142	0.009	-1.293	0.295	-0.045	0.006	-1.326
13	Ala	-0.481	-0.972	0.009	0.581	-0.555	-1.287	0.013	0.468	-0.410	-0.768	0.006	0.415	-0.517	-1.088	0.010	0.448
14	Asn	-0.189	-0.917	0.013	-0.101	-0.368	-1.617	0.022	0.001	-0.233	-0.929	0.012	-0.021	-0.351	-1.302	0.016	0.035
15	Asp	-0.508	-1.179	0.012	0.612	-0.611	-1.291	0.012	0.594	-0.584	-1.052	0.008	0.788	-0.607	-1.324	0.012	0.631
16	Glu	-0.653	-1.103	0.008	0.966	-0.724	-1.429	0.012	0.846	-0.399	-0.971	0.010	0.364	-0.685	-1.314	0.011	0.814

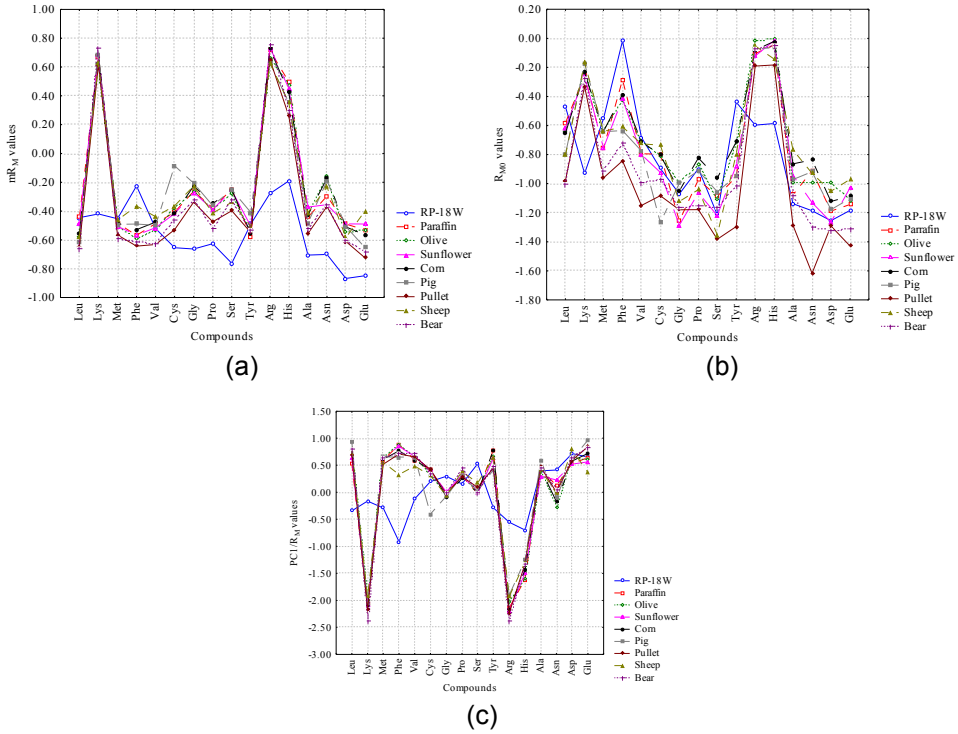


Figure 2. The correlation patterns of lipophilicity indices ((a) mR_M; (b) R_{M0}; (c) $PC1/R_M$) corresponding to the investigated stationary phases.

In order to evaluate the suitability of oils and animal fats impregnated plates as reversed phase for TLC determination of amino acids lipophilicity, the obtained results were compared with a series of theoretical lipophilicity indices. The correlation matrix of the experimental values versus theoretical ones is characterized by reasonable correlation coefficients (Table 5) in case of R_{M0} and b lipophilicity parameters in all cases. These correlations show in all cases that the specific surface area (b) of stationary phases is also a good alternative descriptor of amino acids lipophilicity. The mean (mR_M) of retention parameter R_M proved to be a good lipophilicity parameter only in case of RP-18 stationary phases ($r_{log\ Pow}$ = 0.92 and r_{ALOGPs} = 0.95) having low statistical significance in case of all impregnated stationary phases. By a careful examination, it can observe that in case of pig and respectively sheep fat impregnated stationary phases the correlations between theoretical and experimental lipophilicity indices are not statistically significant. Among the used different calculated lipophilicity indices, the best correlations were obtained with ALOGPs, log P_{ow} and respectively XLOGP3 values for all the RP-18W,

oils and respectively pullet and bear fat impregnated stationary phases. These correlations illustrate that the substructure of molecule and both topological and valence states of atoms have an important contribution on the lipophilicity of the amino acids compounds.

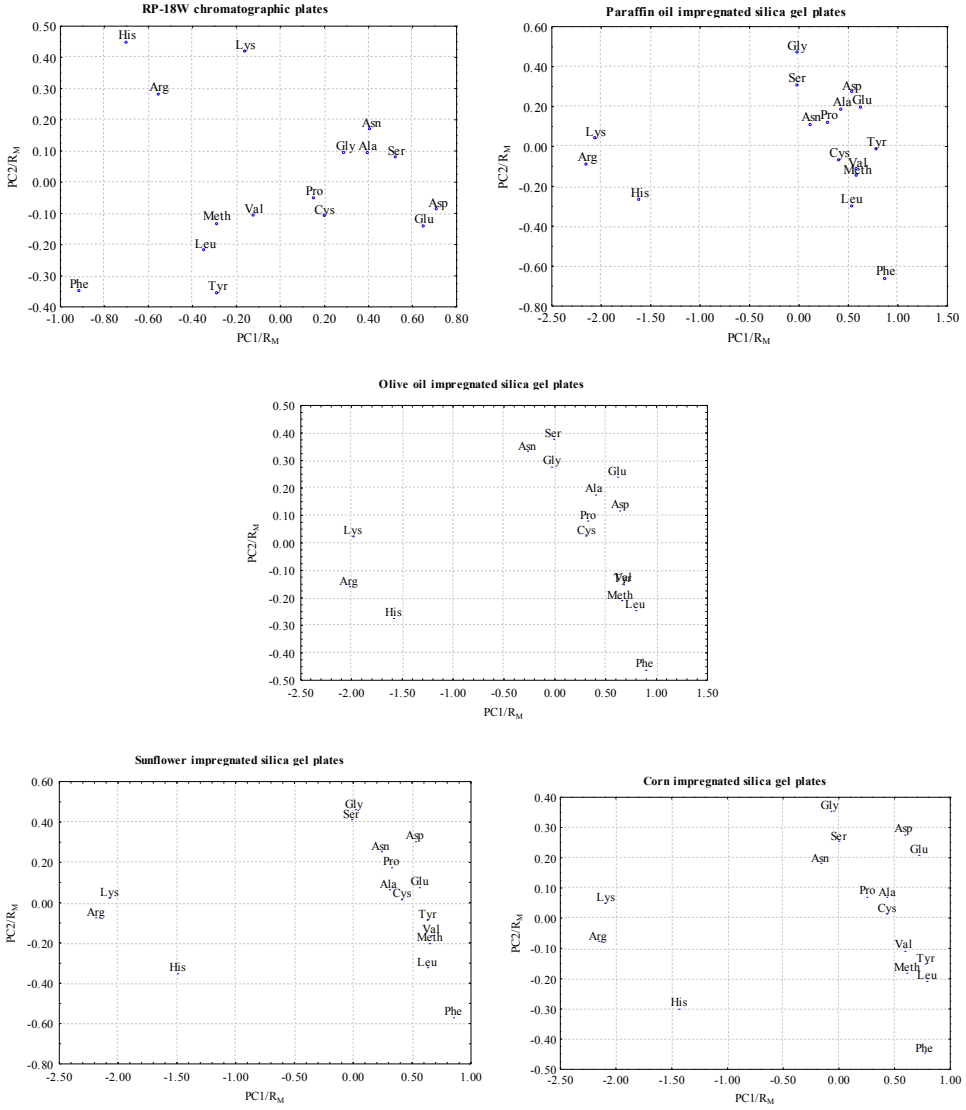


Figure 3a. Lipophilicity charts corresponding to R_M values in case of RP-18W and different oils impregnated silica gel plates.

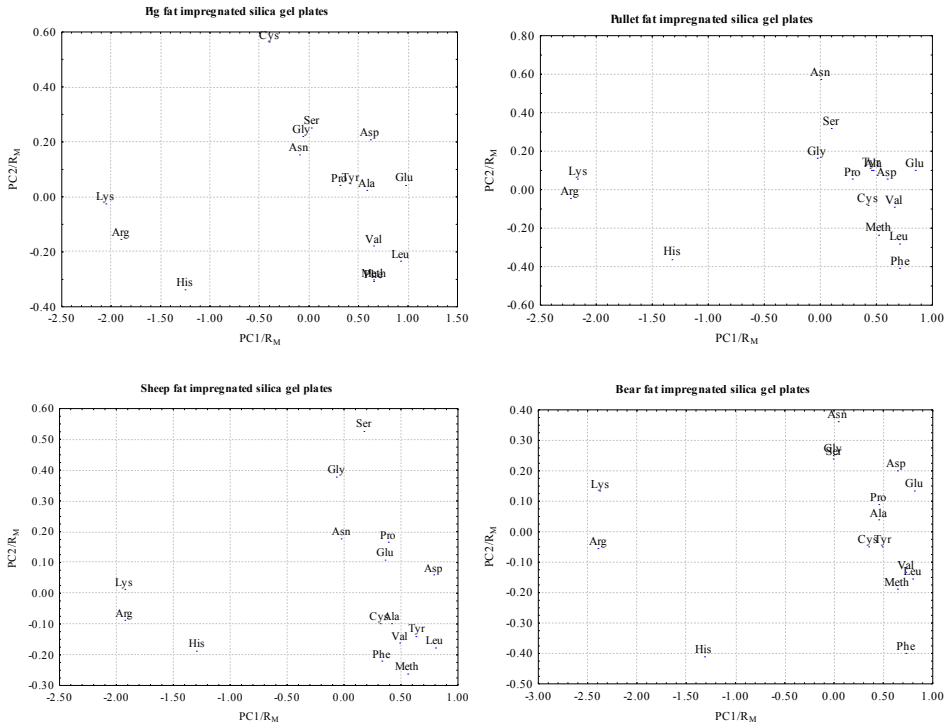


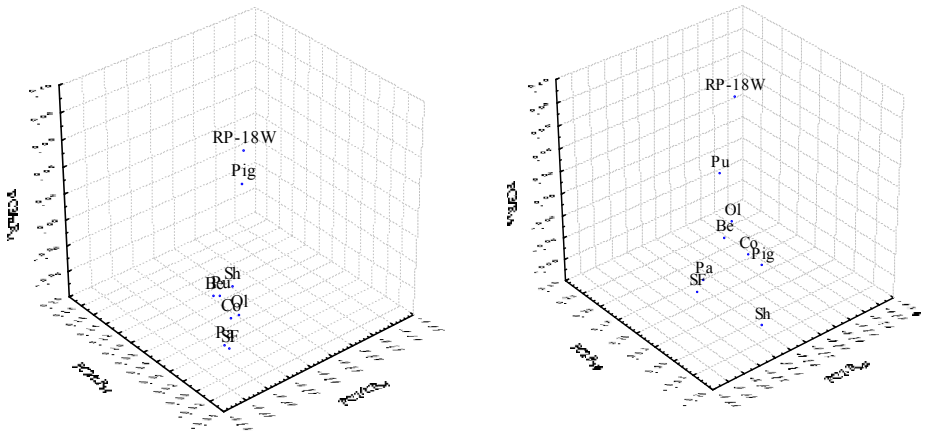
Figure 3b. Lipophilicity charts corresponding to R_M values in case of different animal fats impregnated silica gel plates.

Statistically significant correlations were obtained also with solubility parameter AClogS for almost of the used stationary phases, exception being in case of pig and pullet fat impregnated stationary phases (Table 5). These results indicate that newly ALOGPs version of log P computing module, based on associative neutral networks method, seems to cover, in the most efficiently way, the lipophilic character of amino acids.

Surprisingly, the log D and log P_n values, calculated by correctly adjust for charged parts of molecules are not so well statistically correlated with experimental lipophilicity indices in some cases. The best correlations coefficients are r = 0.91 and r = -0.93 between log P_n and $R_{M0RP18W}$ and respectively $b_{RP\text{-}18W}$; r = -0.91 between log P_n and b_{bear}; r = 0.92 and r = -0.94 between log D(IP) and $R_{M0RP18W}$ and respectively $b_{RP\text{-}18W}$; r = -0.92 between log D(IP) and b_{corn}; r = -0.92 between log D(IP) and b_{bear}.

These results are in good agreement with the properties of amino acids that having both amine and carboxylic acid functional groups, at a certain pH (known as isoelectric point -IP) they can have both positive and negative

charges (zwitterions). Amino acids can exist as zwitterions in polar solutions such as water [20] this fact being supported by the corelations of experimental lipophilicity indices and log D(IP) values in most of the cases. In order to getting more information concerning the similarities and differences between the oils and animal fats impregnated layers, PCA was applied to the matrices resulted by considering the experimental lipopilicity indices mR_M and respectively R_{M0} obtained for all RP-18W and impregnated stationary phases. According to the "lipophilicity space" obtained by 3D representation of scores corresponding to the first three principal components (Figure 4), the RP-18W stationary phases lipophilicity appears in the group of outliers including pig fat (in case of mR_M values) and respectively pullet fat (in case of R_{M0} values) impregnated silica gel plates. The different lipophilicity of RP-18W stationary phase is very well supported also by PC1/R_M representations provided by Figure 2.



(a) (b)

Figure 4. The "lipophilicity space" provided by score plots of the first three principal components (PC1, PC2, PC3) obtained by applying PCA on the: (a) mR_M lipophilicity indices; (b) R_{M0} lipophilicity indices.

Table 5. The correlation between the theoretical and experimental lipophilicity indices of amino acids (Marked correlations are statistically significant)

Stationary Phases	Lipophilicity indices	log P_{ow}	log P_n	log D (pH=7)	log D (IP)	ClogP	ALOGP	MLOGP	ALOGPs	AC log P	miLogP	XLOGP2	XLOGP3	S_{exp}	ALOGpS	AClogS
RP-18W	mR_M	**0.92**	**0.83**	**0.89**	**0.84**	0.71	**0.83**	0.69	**0.95**	0.70	**0.89**	0.68	**0.86**	-0.29	-0.63	**-0.84**
	R_{M0}	**0.91**	**0.91**	**0.84**	**0.92**	**0.82**	**0.91**	0.74	**0.96**	0.76	**0.88**	0.72	**0.89**	-0.39	-0.76	**-0.92**
	b	**-0.86**	**-0.93**	-0.76	**-0.94**	**-0.87**	**-0.92**	-0.75	**-0.91**	-0.77	**-0.82**	-0.71	**-0.86**	0.46	0.83	**0.93**
	PC1/R_M	**-0.92**	**-0.87**	**-0.88**	**-0.88**	-0.75	**-0.87**	-0.72	**-0.96**	-0.73	**-0.89**	-0.70	**-0.88**	0.33	0.68	**0.88**
Paraffin Oil	mR_M	-0.41	-0.73	-0.28	-0.74	-0.66	-0.67	-0.52	-0.52	-0.49	-0.42	-0.45	-0.49	0.73	0.73	0.71
	R_{M0}	**0.82**	0.69	0.70	0.71	0.73	0.70	0.58	**0.91**	0.58	0.73	0.55	**0.82**	-0.32	-0.68	**-0.83**
	b	**-0.81**	**-0.81**	-0.66	**-0.82**	**-0.81**	-0.79	-0.64	**-0.91**	-0.63	-0.74	-0.60	**-0.83**	0.50	0.79	**0.91**
	PC1/R_M	0.52	0.79	0.38	0.79	0.72	0.73	0.57	0.63	0.55	0.51	0.50	0.58	-0.72	-0.78	-0.79
Olive Oil	mR_M	-0.54	-0.77	-0.32	-0.77	**-0.81**	-0.73	-0.57	-0.59	-0.55	-0.45	-0.55	-0.63	0.59	0.57	0.66
	R_{M0}	**0.91**	**0.88**	**0.84**	**0.89**	**0.83**	**0.86**	0.71	**0.98**	0.70	**0.86**	0.71	**0.94**	-0.41	-0.72	**-0.92**
	b	**-0.85**	**-0.93**	-0.71	**-0.94**	**-0.92**	**-0.91**	-0.73	**-0.92**	-0.72	-0.78	-0.72	**-0.91**	0.54	0.74	0.91
	PC1/R_M	0.60	**0.81**	0.39	**0.81**	**0.85**	0.78	0.61	0.65	0.59	0.51	0.59	0.69	-0.60	-0.61	-0.71
Sunflower oil	mR_M	-0.49	-0.73	-0.32	-0.74	-0.76	-0.68	-0.55	-0.65	-0.45	-0.51	-0.48	-0.62	0.80	0.76	0.77
	R_{M0}	**0.88**	**0.84**	0.73	**0.86**	0.78	**0.84**	0.65	**0.93**	0.69	0.78	0.64	**0.82**	-0.43	-0.70	**-0.90**
	b	**-0.82**	**-0.86**	-0.66	**-0.87**	**-0.82**	**-0.84**	-0.66	**-0.90**	-0.66	-0.75	-0.63	**-0.81**	0.56	0.76	**0.91**
	PC1/R_M	0.58	0.78	0.40	0.78	**0.80**	0.73	0.59	0.72	0.51	0.58	0.53	0.68	-0.77	-0.78	**-0.82**
Corn Oil	mR_M	-0.46	-0.71	-0.24	-0.71	-0.74	-0.68	-0.48	-0.51	-0.54	-0.37	-0.47	-0.53	0.61	0.62	0.65
	R_{M0}	**0.89**	0.78	**0.86**	0.79	0.68	0.77	0.67	**0.94**	0.64	**0.83**	0.64	**0.84**	-0.29	-0.66	**-0.86**

Stationary Phases	Lipophilicity indices	log P_{ow}	log P_n	log D (pH=7)	log D (IP)	ClogP	ALOGP	MLOGP	ALOGPs	AC log P	miLogP	XLOGP2	XLOGP3	S_{exp}	ALOGpS	AClogS
	b	**-0.87**	**-0.91**	-0.75	**-0.92**	**-0.85**	**-0.89**	-0.72	**-0.94**	-0.73	-0.78	-0.70	**-0.87**	0.50	0.78	**0.95**
	PC1/R_M	0.54	0.77	0.33	0.78	0.79	0.74	0.54	0.60	0.59	0.45	0.53	0.61	-0.61	-0.67	-0.72
Pig Fat	mR_M	-0.24	-0.45	-0.01	-0.45	-0.49	-0.44	-0.37	-0.28	-0.30	-0.25	-0.31	-0.30	0.66	0.21	0.28
	R_{M0}	0.69	0.60	0.64	0.60	0.51	0.58	0.57	0.75	0.42	0.72	0.52	0.65	-0.40	-0.31	-0.53
	b	-0.58	-0.63	-0.41	-0.64	-0.60	-0.61	-0.56	-0.63	-0.44	-0.59	-0.50	-0.58	0.62	0.32	0.49
	PC1/R_M	0.29	0.48	0.05	0.48	0.51	0.46	0.39	0.33	0.32	0.29	0.34	0.34	-0.67	-0.22	-0.30
Pullet Fat	mR_M	-0.33	-0.56	-0.07	-0.57	-0.64	-0.53	-0.38	-0.40	-0.34	-0.25	-0.35	-0.39	0.61	0.49	0.53
	R_{M0}	**0.86**	0.76	0.69	0.77	**0.83**	0.75	0.61	**0.85**	0.71	0.67	0.71	**0.86**	-0.08	-0.46	-0.72
	b	-0.79	**-0.82**	-0.55	**-0.82**	**-0.90**	-0.79	-0.62	**-0.82**	-0.69	-0.61	-0.68	**-0.82**	0.32	0.55	0.77
	PC1/R_M	0.38	0.60	0.12	0.60	0.68	0.57	0.41	0.45	0.38	0.29	0.39	0.44	-0.60	-0.51	-0.57
Sheep Fat	mR_M	-0.31	-0.48	-0.15	-0.48	-0.64	-0.47	-0.38	-0.33	-0.39	-0.21	-0.41	-0.53	0.48	0.31	0.38
	R_{M0}	0.65	0.71	0.60	0.73	0.56	0.66	0.47	0.77	0.50	0.64	0.48	0.64	-0.55	-0.67	**-0.83**
	b	-0.66	-0.77	-0.55	-0.78	-0.72	-0.73	-0.54	-0.76	-0.56	-0.61	-0.56	-0.73	0.64	0.66	**0.83**
	PC1/R_M	0.37	0.54	0.21	0.54	0.68	0.52	0.42	0.39	0.43	0.27	0.44	0.57	-0.52	-0.37	-0.45
Bear Fat	mR_M	-0.39	-0.63	-0.14	-0.62	-0.69	-0.60	-0.52	-0.48	-0.41	-0.38	-0.49	-0.48	0.71	0.46	0.57
	R_{M0}	**0.93**	**0.82**	**0.88**	**0.83**	0.73	0.79	0.59	**0.92**	0.68	0.73	0.63	**0.84**	-0.05	-0.62	**-0.83**
	b	**-0.88**	**-0.91**	**-0.71**	**-0.92**	**-0.88**	**-0.88**	-0.69	**-0.91**	-0.70	-0.72	-0.71	**-0.86**	0.40	0.68	**0.90**
	PC1/R_M	0.45	0.67	0.20	0.66	0.73	0.64	0.55	0.53	0.45	0.43	0.52	0.53	-0.69	-0.49	-0.61

CONCLUSIONS

Different lipophilicity indices of amino acids on RP-18W and different oils (paraffin, olive, sunflower and corn) and respectively animal fats (pig, pullet, sheep and bear) impregnated silica gel plates were determined using methanol-phosphate buffer as mobile phase. The obtained results indicate no significant differences, in terms of lipophilicity, between oils and animal fats impregnated silica gel plates. The correlation between the theoretical and chromatographic lipophilicity indices revealed that all oils and some animal fats (pullet and bear) can be a good alternative in the field of chromatographic lipophilicity estimation of amino acids. From the lipophilicity used indices, the R_{M0} and b values showed, in all cases, the most significant correlations. The PCA methodology proved to be again a useful tool that can offer a realistic characterization of impregnated plates, both from the retention mechanism and lipophilicity point of view.

EXPERIMENTAL SECTION

Chemicals

The amino acids Leucine (Leu), Lysine (Lys), Methionine (Met), Phenylalanine (Phe), Valine (Val), Cysteine (Cys), Glycine (Gly), Proline (Pro), Serine (Ser), Tyrosine (Tyr), Arginine (Arg), Histidine (His), Alanine (Ala), Asparagine (Asn), Aspartic acid (Asp) and Glutamic acid (Glu) of analytical grade were obtained from Merck or Fluka. Analytical - grade methanol was purchased from Chemical Company (Iasi, Romania). The oils (paraffin, olive, sunflower and corn) and fats (from pig, pullet, sheep and bear), used for silica gel plates impregnation, were from local markets. Ninhydrin, used as visualization reagent, was from Riedel-de Haen (Seelze, Germany).

Thin-Layer Chromatography

The chromatographic behavior of series of amino acids compounds was studied on eight different impregnated silica gel layers (10 x 20 cm) and on RP-18W (10 X 20 cm) chromatographic plates. The silica gel plates were impregnated with 10% of oil and respectively 5% of animal fat in diethyl ether solution in all cases, by ascendant development. The animal fats used as raw material were extracted from the natural membranes by heating to melting point followed by filtration. The standard solutions of amino acids (2 mg/mL) were prepared in methanol and respectively water and 2µL of which were applied manually, in duplicate, on the plate by means of a 10 µL Hamilton (Switzerland) microliter syringe. The mobile phase consisting of different proportions of methanol and phosphate buffer (pH = 7) mixture was from

20% to 90% methanol in all cases. Chromatography was performed in a normal developing chamber, saturated for 15 min at room temperature (~22 ^{0}C), by ascendant development and a developing distance of 8 cm in all cases. The amino acids were visualized by using a 0.2% ninhydrin solution prepared in ethanol and heating the plates at 110 ^{0}C for 10 minutes.

Computation of lipophilicity indices

Nowadays, it is well known that many software and internet modules are able to calculate different lipophilicity values applying various algorithms based on structural, atomistic, topological or electrotopological considerations. All of them require a previously molecule drawing that is usually performed by Hyperchem [21] and optimized using the MM+ molecular mechanics force field. On the basis of obtained geometry, software like Chem3D Ultra 8.0 [22] and Dragon Plus version 5.4 [23] calculate various lipophilicity descriptors. In the present study, one log P value (Clog P) was calculated by Chem3D Ultra and two log P values (MLOGP-Moriguchi method and ALOGP- Ghose-Crippen method) by the Dragon Plus software. Another five lipophilicity descriptors (ALOGPs, AC logP, miLogP, XLOGP2, XLOGP3) were computed by the internet module ALOGPS 2.1 [24]. By using this free internet module we derived also a set of three solubility indices (ALOGpS, AC logS, AB/logS). The experimental solubility in water and octanol-water partition coefficient of studied compounds are from the Human Metabolome Project database [25].

In some cases, the distribution coefficient (log D) of a compound at a given pH may be used as an appropriate descriptor for lipophilicity estimation. Because of the nature of studied compounds, we derived log D values for two different pH (log D (pH = 7) and log D (PI) -at isoelectric point of each compound) and respectively log P for nonionic species of amino acids (log P_n) by using a new and improved log P calculator available as free internet module Marvin Sketch 5.3.2 [26].

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