Quantitative Structure–Liquid Chromatographic Retention Time Relationships on Natural Phenols Found in Olive Oil

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ABSTRACT: A study of the relationship between the liquid chromatographic retention times for a group of 16 natural phenols on olive oil and an initial set of 62 molecular descriptors has been made. The descriptors used may be arranged into three well-defined kinds: conventional, topological, and quantum-chemical parameters. By using multivariate regression, three empirical functions were obtained, which were selected on the basis of their respective statistical parameters. The first model relates the retention index with the quantum-chemical descriptors; the second, with both topological and conventional descriptors; and finally, the third model has been established by considering the relation of the experimental magnitude with the two above-mentioned sets of parameters together. The correlation coefficients of the empirical functions were 0.9825, 0.9911, and 0.9974, with mean relative errors of 6.8, 4.6, and 2.6%, respectively.

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KEY WORDS: Olive oil, phenols, quantitative structure–activity relationships, quantitative structure–retention time relationships.

Phenolic compounds constitute an important group of naturally occurring compounds in plants. In contrast to other crude oils, virgin olive oil produced from olives of good quality is consumed unrefined. Thus, virgin olive oils contain phenolic compounds that are usually removed from other edible oils in the various refining stages (1). Olive oils are low in tocopherols (2); therefore, the presence of other phenolic compounds capable of antioxidant activity is of particular importance (3). Phenols make up a part of the "polar fraction" of virgin olive oil, which is usually obtained by extraction with methanol/water mixtures (4). The presence of phenolic compounds identified in olive oils, such as hydroxytyrosol, tyrosol, caffeic acid, ferulic acid, *p*-coumaric acid, *p*-hydroxyphenylacetic acid, protocatechuic acid, syringic acid, vanillic acid, salicylic acid, and *p*-hydroxybenzoic acid, has been reported previously (5); however, this fraction of the oil is complex and many of these components remain unidentified (6). There is an interest in the level of phenols in olives, olive oil, and the so-called rape, a major by-product of the extraction process, because of the antioxidant activity of the total phenolic fraction (7). Little is known, however, about the contribution of each component to the stability of the oil. High-performance liquid chromatography (HPLC) procedures are difficult to perform, owing to the complexity of the phenolic fraction, and demand rather sophisticated gradient elution separations.

This paper is focused to provide an easy method to corroborate the structural identification of single components in these complex mixtures, as well as to predict the retention times from quantitative structure–retention relationships. The field of quantitative structure–activity relationships (QSAR) relates molecular structures, described numerically, with chemical, physicochemical, and biological activities (8). The methodology of relating chemical structure with chromatographic retention parameters is known as quantitative structure–retention relationships (QSRR) (9) and has two main goals, the prediction of retention coefficients and the explanation of the chromatographic mechanisms (10). Chromatographic retention is based on interactions between the solute and the stationary phase, and the aim of the present work is to find which of the available topological, geometrical, electronic, and physical descriptors that we computed are related to the retention of the phenols present in the olive oil. The results of these studies show good correlation of topological, geometrical, physicochemical, and electrical descriptors vs. retention in chromatographic columns.

EXPERIMENTAL PROCEDURES

Experimental liquid chromatographic retention times (hereafter denoted as TR), and the corresponding experimental details, for the 16 natural phenols used in this work have been taken from the literature (11,12). In Table 1, a relation of the phenols used together with the experimental and calculated TR values is given.

The procedure used in the present study comprised two fundamental stages: (i) generation of molecular descriptors and (ii) statistical analysis.

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TABLE 1
Values of Experimental and Calculated Retention Times (min) from the Three Proposed Models

Compound	TR (exp)	TR (1)	Δ (%)[a]	TR (2)	Δ (%)	TR (3)	Δ (%)
Caffeic acid	14.467	16.282	−11.15	14.697	−1.57	14.781	−2.12
Ferulic acid	23.150	22.893	1.12	22.709	1.94	23.365	−0.92
Gallic acid	4.200	5.947	−29.38	4.575	−8.19	4.512	−6.92
p-Coumaric acid	19.500	17.134	13.81	19.564	−0.33	19.140	1.88
p-Hydroxyphenylacetic acid	11.000	12.021	−8.49	10.107	8.84	10.871	1.18
Protocatechuic acid	7.300	6.736	8.37	6.527	11.85	6.657	9.66
Sinapic acid	27.017	27.240	−0.82	26.997	0.07	26.799	0.81
Syringic acid	15.000	13.852	8.29	15.828	−5.23	14.771	1.55
Vanillic acid	13.700	11.275	21.50	12.496	9.63	13.321	2.85
Veratric acid	22.050	22.407	−1.59	21.180	4.11	22.528	−2.12
Salicylic acid	18.250	17.421	4.76	18.696	−2.38	18.015	1.30
p-Hydroxybenzoic acid	9.883	9.516	3.86	10.602	−6.78	10.789	−8.40
p-Hydroxybenzaldehyde	12.250	13.301	−7.90	12.402	−1.22	12.373	−1.00
4-Hydroxybenzylalcohol	5.800	5.423	6.95	6.583	−11.90	5.920	−2.03
Vanillin	15.417	15.852	−2.74	14.231	8.34	14.595	5.63
Syringaldehyde	16.350	17.412	−6.10	18.149	−9.91	16.893	−3.22

[a] $\Delta(\%) = \dfrac{TR_{exp.} - TR_{calc.}}{TR_{calc.}} \times 100$, where TR = retention time.

Generation of molecular descriptors. Three types of descriptors have been used: conventional, topological, and quantum-chemical descriptors. Both conventional and topological descriptors were calculated by means of the DESCRIPTOR program developed by us for a PC system and GW-BASIC language. Conventional descriptors are basically related with the number and types of atoms and bonds displayed in each molecule. Topological descriptors include valence and nonvalence molecular connectivity indices, calculated from the formula of suppressed hydrogens of the molecule according to the method proposed by Kier and Hall (13,14), and encode information about the size and the degree of branching of a molecule. Quantum-chemical descriptors include information about binding and formation energies, energy levels in the molecule, vibrational energies, and inertia moments. To obtain quantum-chemical descriptors, the semi-empirical method AM1, as implemented in the HYPERCHEM 4.0 software package, was used. All calculations have been carried out at RHF level for the singlet lowest energy state with no configuration interaction. The molecular structures were generated with the molecular builder inside HYPERCHEM and optimized by following the Polak-Ribiere algorithm until RMS gradient 0.1 kcal·Å^{-1}·mol^{-1}. To check the goodness of the resulting structures, the infrared spectrum also has been calculated, and the nonappearance of negative frequencies was assumed to be unequivocal evidence that the generated structure represents a global potential energy minimum, not a local minimum.

Statistical analysis. A total of 62 molecular descriptors have been used to explain the behavior of the dependent variable liquid chromatographic TR. In a first step, two set of descriptors were considered. The first set includes the quantum-chemical descriptors, and the second is constituted of the conventional and topological descriptors. In both sets, a stepwise regression was carried out to select the best independent variables subset, following as criterion a minimum value for the Mallows' C_p. For this purpose, the multicolinearity effect inside each set was eliminated. From this, we considered the following independent variables, whose definitions are given in Table 2.

Set 1: HOMO, LUMO, ENLACE, FORMACIO, MAXPOB, MENCAR, SP, IY, ACM, CCM, MAYVIB, MENVIB

Set 2: NC, N_CH, C1_PESO, CH_ENL, CX_ENL, C1, $^4\chi_p^{\,v}$, $^0\chi_0$, $^3\chi_c^{\,v}$

Then, with the 9R program in the BMDP statistical package, the best subset of regression may be selected. The data and statistics obtained for each set are given in Tables 3 and 4.

If both sets are studied simultaneously, and once the variables that show multicolinearity are removed, the best regression equation given in Table 5 is obtained.

RESULTS AND DISCUSSION

In model 1, whose statistics are given in Table 3, all coefficients are significant above the 99% level, except the MAXPOB index, which is significant above 95%. In model 2 (Table 4), all coefficients are significant above the 99.5% level. Finally, in model 3 (Table 5), all coefficients are significant above the 99.5% level, with the exception of the ENLACE coefficient, which is significant above 98.7%. Plots of experimental vs. expected values of 16 cases, for each regression equation, are depicted in Figures 1 to 3.

Residuals vs. experimental TR values, following the three models, have been plotted in Figure 4. The residuals are normally distributed and independent, and there is no autocorrelation between them.

TABLE 2
Definition of the Total Set of Descriptors Used

Descriptor code	Definition
Quantum-chemical descriptors	
HOMO	HOMO energy (eV)
LUMO	LUMO energy (eV)
ENLACE	Binding energy (kcal·mol^{-1})
FORMACIO	Heat of formation (kcal/mol)
MAXPOB	Highest atomic orbital electron population
MENCAR	Lowest net atomic charge (electron units)
SP	Dipole moment component due to sp lone pairs
IY	Inertia moment in the y axis (g·cm^2·10^{-40})
ACM	Rotational constant in the x axis (cm^{-1})
CCM	Rotational constant in the z axis (cm^{-1})
MAYVIB	Highest vibration frequency in the calculated infrared spectrum (cm^{-1})
MENVIB	Lowest vibration frequency in the calculated infrared spectrum (cm^{-1})
Conventional descriptors	
NC	Number of carbon atoms
N_CH	Number of C–H bonds
C1_PESO	Relative weight of C atoms
CH_ENL	Ratio between number of C–H bonds and total number of bonds
CX_ENL	Ratio number of C–X bonds/total bonds (X = non-H and non-C atoms)
C1	Relative number of carbon atoms
Topological descriptors	
${}^4\chi_p{}^v$	Valence-corrected molecular connectivity, path 4 (A-B-C-D)
${}^3\chi_c{}^v$	Valence-corrected molecular connectivity, cluster 3 (A-$\overset{\overset{\text{D}}{\vert}}{\text{B}}$-C)
${}^0\chi_0$	Zero-order molecular connectivity

In the same way, the Mahalanobis distance shows that extremely high values do not exist, at a confidence level of 95%. If we consider Cook's distance about the influence of a sample value, in the first block, the case number 14 (4-hydroxybenzylalcohol) presents the highest value, while in other regression equations, distances do not appear that show the presence of sample values with a significant influence (especially in the second regression equation). The largest standardized residuals in absolute value among cases are 1.82, 1.65, and 1.64, respectively, for each regression equation.

In conclusion, the first model clearly makes worse predictions than the other two. Also, if a compromise between the exactitude of the predictions and the total number of descriptors used must be found, the second model could be useful and preferable over the third because, despite yielding a little less exact results than those obtained from model 3, the smaller number of variables in the equation will allow easier application.

However, the lack of reproducibility of the chromatographic columns may be a major problem in applying the results reported here; obviously, these models are valid only with the same experimental conditions in which the TR values (from which the statistical models have been calculated) have been measured. Finally, the prediction of TR for new phenol compounds will also depend on the degree of similarity between the query molecules and those in the data set.

SUPPLEMENTARY MATERIAL

Tables with the values of the used descriptors for each of the studied phenols and the corresponding correlation matrix follow.

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REFERENCES

1. Vasquez, R.A., C. Janer del Valle, and M.L. Janer del Valle, Componentes Fenólicos de la Aceituna. III. Polifenoles del Aceite, *Grasas Aceites 27*:185–191 (1976).
2. Gutfinger, T., Polyphenols in Olive Oil, *J. Am. Oil Chem. Soc. 58*:966–968 (1981).
3. Gutfinger, T., and A. Letan, Studies on Unsaponifiables in Several Vegetable Oils, *Lipids 9*:658–663 (1974).
4. Vasquez, R.A., C. Janer del Valle, and M.L. Janer del Valle, Determinación de los Polifenoles Totales del Aceite de Oliva, *Grasas Aceites 24*:350–357 (1973).
5. Akasbi, M., D.W. Shoeman, and A.S. Csallany, High-Performance Liquid Chromatography of Selected Phenolic Compounds in Olive Oils, *J. Am. Oil Chem. Soc. 70*:367–370 (1993).
6. Papadopoulos, G., and D. Boskou, Antioxidant Effect of Natural Phenols on Olive Oil, *J. Am. Oil Chem. Soc. 68*:669–671 (1991).
7. Sheabar, F., and I. Neeman, Separation and Concentration of

TABLE 3
Regression Model 1[a]

Variable	Regression coefficient	Standard error	Contribution to R-SQ
ENLACE	−0.00117	0.00401	0.03718
FORMACIO	0.01021	0.01998	0.11349
MAXPOB	−79.4436	36.0357	0.02110
IY	0.00606	0.00154	0.06744
ACM	−102.482	26.9250	0.06289
CCM	692.826	197.531	0.05341
MAYVIB	−0.05818	0.01626	0.05561
Intercept	344.483	62.7211	

[a] n = 16; Mallows' C_p = 5.10; mean relative error = 6.8%; R = 0.9825; R^2 = 0.9653; $F(7,8)$ = 31.76. For explanation of variables see Table 2.

TABLE 4
Regression Model 2[a]

Variable	Regression coefficient	Standard error	Contribution to R-SQ
C1_PESO	194.009	39.6991	0.04220
CH_ENL	−117.603	12.3679	0.15976
CX_ENL	101.886	29.9562	0.02044
${}^4\chi_p^v$	83.214	5.5878	0.39186
${}^3\chi_c^v$	−178.788	19.5273	0.14812
Intercept	−110.983	28.1963	—

[a] n = 16; Mallows' C_p = 3.83; mean relative error = 4.6%; R = 0.99113; R^2 = 0.98233; $F(5,10)$ = 111.19. For explanation of variables see Table 2.

TABLE 5
Regression Model 3[a]

Variable	Regression coefficient	Standard error	Contribution to R-SQ
ENLACE	0.01868	0.0059	0.00649
IY	0.00425	0.0010	0.01232
C1_PESO	163.484	29.4707	0.02005
CH_ENL	−94.9875	11.1521	0.04728
CX_ENL	102.677	18.9882	0.01906
${}^4\chi_p^v$	98.8128	9.96257	0.06411
${}^3\chi_c^v$	−176.922	12.3750	0.13320
Intercept	−78.7436	20.1406	—

[a] n = 16; Mallows' C_p = 4.21; mean relative error = 2.6%; R = 0.99739; R^2 = 0.99479; $F(7,8)$ = 218.07. For explanation of variables see Table 2.

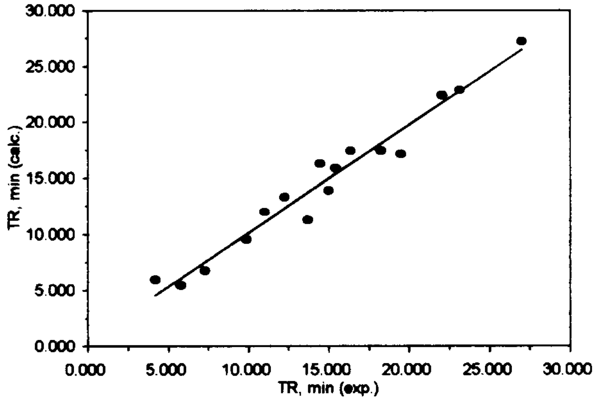

FIG. 1. Plot of experimental vs. expected values of retention time (TR) (model 1).

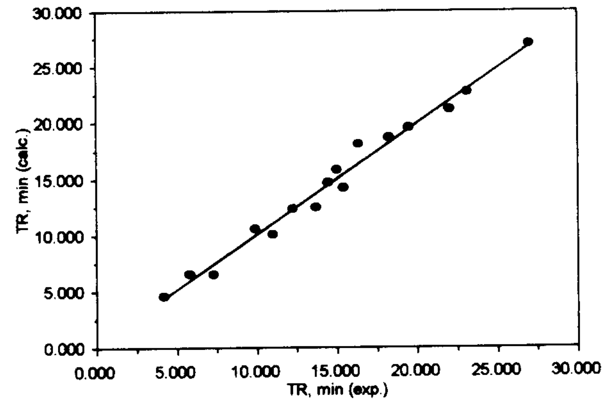

FIG. 2. Plot of experimental vs. expected values of TR (model 2). See Figure 1 for abbreviation.

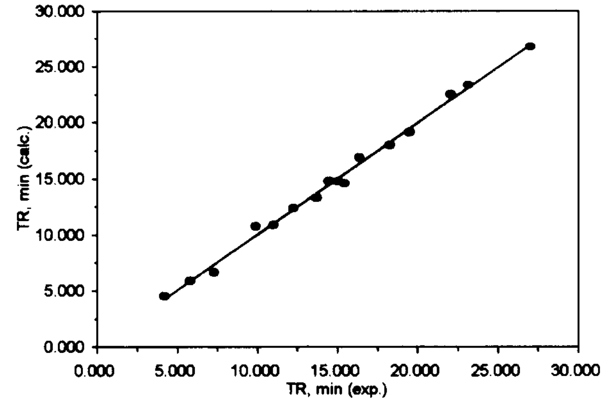

FIG. 3. Plot of experimental vs. expected values of TR (model 3). See Figure 1 for abbreviation.

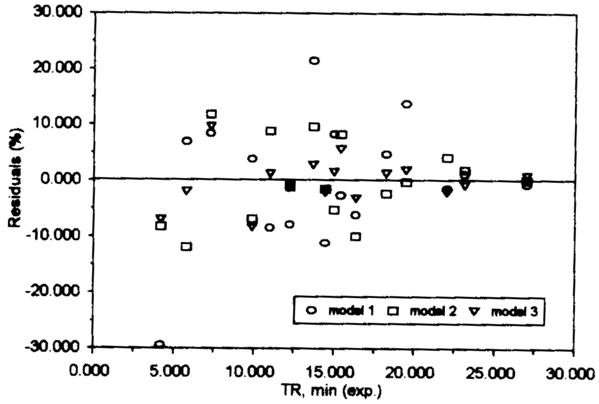

FIG. 4. Plot of residuals vs. experimental values of TR for the three models. See Figure 1 for abbreviation.

TABLE S-1
Quantum-Chemical Descriptor Values for the Phenols Studied

Compound	HOMO	LUMO	ENLACE	FORMACIO	MAXPOB	MENCAR	SP	IY	ACM	CCM	MAYVIB	MENVIB
Caffeic acid	−9.130	−.903	−2331.56	−138.500	1.926	−.362	.615	2587.120	.074	.009	3490.890	49.37
Ferulic acid	−8.994	−.807	−2597.30	−129.140	1.916	−.036	.535	2954.100	.054	.008	3517.120	47.03
Gallic acid	−9.404	−.707	−1998.57	−191.930	1.922	−.357	.151	1281.910	.052	.015	3484.170	35.17
p-Coumaric acid	−9.117	−.805	−2232.68	−99.170	1.915	−.364	.772	2372.140	.120	.011	3451.000	51.74
p-Hydroxyphenylacetic acid	−9.143	.162	−2078.91	−116.290	1.916	−.374	.983	1553.570	.115	.016	3458.020	58.54
Protocatechuic acid	−9.466	−.625	−1898.88	−151.800	1.927	−.362	.636	1206.260	.081	.018	3489.260	39.13
Sinapic acid	−8.872	−.820	−2965.89	−163.080	1.917	−.361	.433	3227.810	.026	.007	3516.130	35.51
Syringic acid	−9.144	−.504	−2533.15	−176.330	1.915	−.361	.444	1525.730	.027	.011	3511.240	32.50
Vanillic acid	−9.281	−.499	−2164.65	−142.470	1.915	−.364	.553	1497.730	.065	.015	3513.100	34.08
Veratric acid	−9.117	−.389	−2420.55	−123.280	1.915	−.366	.329	1838.160	.056	.012	3428.900	38.49
Salicylic acid	−9.462	−.591	−1802.07	−114.554	1.916	−.400	1.132	702.692	.077	.026	3417.940	118.88
p-Hydroxybenzoic acid	−9.608	−.482	−1800.62	−113.097	1.915	−.370	.775	1074.104	.129	.022	3449.480	101.49
p-Hydroxybenzaldehyde	−9.490	−.449	−1682.00	−54.040	1.911	−.295	.582	842.990	.168	.028	3449.740	75.30
4-Hydroxybenzylalcohol	−8.912	.392	−1805.25	−73.090	1.975	−.328	.502	868.910	.156	.027	3502.020	6.51
Vanillin	−9.200	−.462	−2046.25	−83.640	1.914	−.289	.889	1116.060	.068	.018	3516.040	47.03
Syringaldehyde	−9.068	−.473	−2414.69	−117.420	1.915	−.286	.528	1142.340	.028	.013	3513.840	34.74

TABLE S-2
Conventional and Topological Descriptor Values for the Phenols Studied

Compound	NC	N_CH	C1_PESO	CH_ENL	CX_ENL	C1	$^4\chi_p^v$	$^0\chi_0$	$^3\chi_c^v$
Caffeic acid	9	5	.6000	.2381	.1905	.4286	.812	9.845	.278
Ferulic acid	10	8	.6185	.3333	.2083	.4167	.965	10.552	.272
Gallic acid	7	2	.4942	.1111	.2778	.3889	.728	9.301	.314
p-Coumaric acid	9	6	.6585	.3000	.1500	.4500	.753	8.975	.223
p-Hydroxyphenylacetic acid	8	6	.6315	.3158	.1579	.4211	.788	8.268	.257
Protocatechuic acid	7	3	.5455	.1765	.2353	.4118	.656	8.431	.258
Sinapic acid	11	10	.5892	.3571	.2500	.3929	1.177	12.129	.323
Syringic acid	9	8	.5455	.3333	.2917	.3750	1.017	10.715	.303
Vanillic acid	8	6	.5714	.3000	.2500	.4000	.811	9.138	.252
Veratric acid	9	9	.5934	.3913	.2609	.3913	.969	9.845	.247
Salicylic acid	7	4	.6087	.2500	.1875	.4375	.654	7.560	.182
p-Hydroxybenzoic acid	7	4	.6087	.2500	.1875	.4375	.604	7.560	.204
p-Hydroxybenzaldehyde	7	5	.6884	.3333	.1333	.4667	.553	6.690	.171
4-Hydroxybenzylalcohol	7	6	.6773	.3529	.1176	.4118	.601	6.690	.192
Vanillin	8	7	.6315	.3684	.2105	.4211	.768	8.268	.220
Syringaldehyde	9	9	.5934	.3913	.2609	.3913	.982	9.845	.270

TABLE S-3
Correlation Matrix for the Descriptors Used[a]

HOMO	1.0000																					
LUMO	0.1459	1.0000																				
ENLACE	–0.7159	0.4155	1.0000																			
FORMACIO	–0.0352	0.4503	0.5029	1.0000																		
MAXPOB	0.3312	0.5881	0.2686	0.2095	1.0000																	
MENCAR	0.3102	–0.1561	–0.2654	0.1558	–0.0466	1.0000																
SP	–0.2678	0.1584	0.3886	0.4698	–0.1737	–0.1470	1.0000															
IY	0.6072	–0.5277	–0.8253	–0.3613	–0.1952	0.3297	–0.2583	1.0000														
ACM	–0.3150	0.4962	0.7445	0.7415	0.3827	–0.1098	0.3445	–0.4000	1.0000													
CCM	–0.5565	0.5308	0.9009	0.6081	0.3544	–0.2177	0.3730	–0.8499	0.7059	1.0000												
MAYVIB	0.5094	–0.0873	–0.4771	–0.3030	0.1927	0.4214	–0.3806	0.3044	–0.4591	–0.4272	1.0000											
MENVIB	–0.6680	–0.2054	0.4193	0.2463	–0.4680	–0.1557	0.6678	–0.2671	0.2775	0.4147	–0.6863	1.0000										
NC	0.7452	–0.4222	–0.9674	–0.3028	–0.2866	0.3403	–0.2578	0.8772	–0.6006	–0.8624	0.4079	–0.3315	1.0000									
N_CH	0.7639	0.0212	–0.7812	0.0402	–0.1460	0.3059	–0.1899	0.4900	–0.4493	–0.5335	0.3619	–0.3770	0.8158	1.0000								
C1_PESO	0.1980	0.3977	0.2703	0.9280	0.2302	0.2056	0.4598	–0.0635	0.7149	0.4054	–0.2462	0.1937	–0.0444	0.1948	1.0000							
CH_ENL	0.6067	0.3221	–0.3987	0.4796	–0.0209	0.2786	0.0550	0.1510	–0.0538	–0.1271	0.1772	–0.2265	0.4966	0.8780	0.5560	1.0000						
CX_ENL	0.0343	–0.4225	–0.5424	–0.8055	–0.3940	–0.0478	–0.5278	0.1683	–0.8953	–0.5548	0.3649	–0.2924	0.3434	0.2554	–0.8700	–0.1042	1.0000					
C1	–0.4128	–0.0354	0.5705	0.7127	–0.0883	0.0379	0.6034	–0.1589	0.7711	0.5021	–0.5262	0.6333	–0.3597	–0.4028	0.7085	–0.0883	–0.8243	1.0000				
$^{4}\chi_p^{v}$	0.6746	–0.3247	–0.9736	–0.5192	–0.3147	0.2122	–0.3814	0.6885	–0.8152	–0.8420	0.4582	–0.4073	0.9109	0.8169	–0.3350	0.4508	0.6424	–0.6735	1.0000			
$^{0}\chi_0$	0.5491	–0.5544	–0.9614	–0.6998	–0.3500	0.1930	–0.4629	0.7864	–0.8446	–0.9267	0.4729	–0.3845	0.8824	0.6092	–0.5060	0.1638	0.7023	–0.6407	0.9414	1.0000		
$^{3}\chi_c^{v}$	0.4046	–0.3962	–0.7591	–0.8679	–0.1938	0.0642	–0.5710	0.5963	–0.7830	–0.8248	0.5501	–0.5127	0.6085	0.2959	–0.7294	–0.1584	0.7445	–0.7533	0.7576	0.8729	1.0000	
TR	0.4905	–0.4539	–0.7439	–0.0307	–0.4593	0.2877	0.0553	0.6751	–0.4605	–0.5665	0.0224	0.0872	0.8330	0.7561	0.1596	0.5712	0.1916	–0.0634	0.7166	0.6358	0.2016	1.0000

[a] For definitions of descriptors see Table 2.

Natural Antioxidants from the Rape of Olives, *J. Am. Oil Chem. Soc. 65*:990–993 (1988).

8. Hansch, C., Quantitative Structure–Activity Relationship in Drug Design, in *Drug Design,* edited by E.J. Ariens, Academic, New York, 1971, Vol. 1, p. 271.
9. Kaliszan, R., *Quantitative Structure–Chromatographic Retention Relationship,* John Wiley & Sons, New York, 1987.
10. Georgakopoulos, C.G., O.G. Tsika, J.C. Kiburis, and P.C. Jurs, Prediction of Gas Chromatographic Relative Retention Times of Anabolic Steroids, *Anal. Chem. 63*:2025–2028 (1991).
11. Martínez, L., S.E. Garrido, F. Camacho, and A. Ramos, The Biological Purification of Waste Products from Olive Oil Extraction, *Biores. Technol. 43*:215–219 (1993).
12. Borja, R., S.E. Garrido, L. Martínez, A. Ramos, and A. Martín, Kinetic Study of Anaerobic Digestion of Olive Mill Wastewater Previously Fermented with *Aspergillus terreus*, *Process Biochem. 28*:397–404 (1993).
13. Randic, M., On Characterization of Molecular Branching, *J. Am. Chem. Soc. 97*:6609–6615 (1975).
14. Kier, L.B., and L.H. Hall, *Molecular Connectivity Analysis,* Wiley, New York, 1986.

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