

Short communication

# Simulation of chromatography of phenolic compounds with a computational chemical method

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## Abstract

An ab initio simulation of reversed-phase liquid chromatography for phenolic compounds was achieved based on molecular interaction energy values calculated using molecular mechanics calculations (MM2) of the CAChe program. The precision of the predicted retention factors from the molecular interaction energy values was equivalent to the predicted retention factors based on octanol–water partition coefficients ( $\log P$ ) calculated using the molecular orbital package (MOPAC). The prediction of retention factors of phenolic compounds in reversed-phase liquid chromatography in a given pH eluent was possible using the predicted dissociation constant ( $pK_a$ ) from the atomic partial charge without a chemical experiment if the organic modifier effect was known.

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**Keywords:** Computer simulation; Structure–retention relationships; Molecular interactions; Retention prediction; Dissociation constants; Octanol–water partition coefficients; Phenolic compounds

## 1. Introduction

An important property in the process of drug discovery is human serum albumin (HSA)–drug candidate binding affinity. Several experimental methods have been applied using HSA, however, these methods are time consuming and show poor reproducibility according to varied binding affinity values ( $\log nK$ ) from different references. Hummel–Dreyer and Frontal analyses have been used to measure protein–drug binding affinity by liquid chromatography [1,2]. The protein binding affinity of drugs was determined using a physically protein-coated ODS column [3] and a chemically bonded bovine serum albumin column [4–7]. The immobilized protein column method is simple but the columns are not stable. The active sites are probably buried by binding reaction. These are fundamental problems for protein–drug binding measurement. Therefore, a new liquid chromatographic system was developed to measure protein–drug binding affinity indirectly without albumin and evaluated using  $\log nK$  values of drugs measured by a modified Hummel–Dreyer method using purified human serum albumin. The retention factors of acidic and basic drugs were measured by

reversed-phase and ion-exchange liquid chromatography in sodium phosphate buffer, pH 7.40, containing 50% (v/v) methanol at 37 °C. The bonded phases were pentyl, guanidino and carboxyl phases. The combined retention factors correlated well with the  $\log nK$  values measured by a modified Hummel–Dreyer method. This liquid chromatographic method was reproducible and faster than the ordinary methods [8]. However, the analysis time in the new liquid chromatographic method cannot compete for the measurement of octanol–water partition coefficient,  $\log P$ , and dissociation constant,  $pK_a$ . Therefore, the rapid analysis should be further developed using a computational chemical method like that used to calculate  $\log P$  values. In computational chemical approaches, Border proposed a method known as  $B \log P$  based on the molecular orbital package (MOPAC) calculation [9]. However, this method is not suitable for larger sized molecules, because the molecular size has a negative effect in the equation. A calculation method based on MOPAC calculation called CAChe  $\log P$  was developed by CAChe Scientific [10]. However, the accuracy of the CAChe  $\log P$  (CA  $\log P$ ) method was not sufficient due to poor correlation between CA  $\log P$  and  $\log P$  values obtained experimentally ( $M \log P$ ) by octanol–water partitioning for phenolic and nitrogen-containing compounds. Several  $\log P$  calculation methods were evaluated by comparison with reference values [9,11], and a new method, a modified CA  $\log P$  method,

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was proposed for optimization of reversed-phase liquid chromatography. The new  $\log P$  values were evaluated with  $\log k$  values of phenolic and nitrogen-containing compounds measured in reversed-phase liquid chromatography [12].

In the process of conversion from a liquid chromatographic method to a computational chemical method, a calculation method to predict the retention time in a given pH eluent should be developed especially to predict the retention time in pH 7.40. Computational chemical analysis is mainly performed in medicinal chemistry and material science. The chemical structure of components is proposed for finding potent drugs having the same mechanism of action [13]. A right inhibitor is selected in the center of the enzyme's catalytic site. Computational chemistry is used to improve and understand chemical reactivity [14]. The theoretical basis for metabolic stability and its relationship to the concept of metabolic intrinsic clearance is studied [15]. Previously, the prediction of retention times in reversed-phase liquid chromatography, quantitative structure–retention relationship (QSRR), was achieved using the octanol–water partition coefficient,  $\log P$  values, based on computational chemical method [16]. The retention time of energetic materials in reversed-phase liquid chromatography was predicted using theoretical linear solvation energy relationships (TLSER). The descriptors were molecular volume, polarizability index, covalent hydrogen bonding basicity, electrostatic hydrogen bonding basicity, covalent hydrogen bonding acidity and electrostatic hydrogen bonding acidity. The regression coefficient ( $r^2$ ) was 0.804 ( $n = 22$ ). The TLSER methodology relied on the semiempirical formulations of molecular orbital theory rather than *ab initio* methods [16a]. Furthermore, an *ab initio* simulation of reversed-phase liquid chromatography for phenolic compounds was performed using

a molecular mechanics calculation (MM2) in molecular mechanics of the CAChe program [10]. A model bonded phase was constructed to study the molecular interactions in reversed-phase liquid chromatography [17,18].

The interaction energy values between a molecular or an ionized form compound and the model butyl-phase were calculated to analyze QSRR. The interaction energy values ( $\Delta$  values) of the final structure (optimized structure) and Van der Waals were well correlated with the  $\log k$  values of molecular and ionized form phenolic compounds, and regression coefficient ( $r^2$ ) values were more than 0.98 for standard compounds where the retention factors were measured using a pentyl-bonded silica gel column in reversed-phase liquid chromatography [19].

The correlation obtained for the molecular forms was used to predict the maximum retention factors of these compounds, and that for the ionized forms was used to predict the minimum retention factors. Furthermore, these retention factors were used to predict retention factors in given pH eluents. The  $k$  values were well correlated with  $\Delta$  values of the final structure or Van der Waals,  $r^2 > 0.85$ – $0.99$  (pH 3–9) [19]. In this study, the retention data measured by reversed-phase liquid chromatography using an octadecyl-bonded phase in various pH eluents [20] were analyzed by a computational chemical method and furthermore a new model phase was constructed to improve the precision.

## 2. Experimental

The computer was Dell model Latitude C840 equipped with a 2 GHz processor and 1024 Mb memory. The

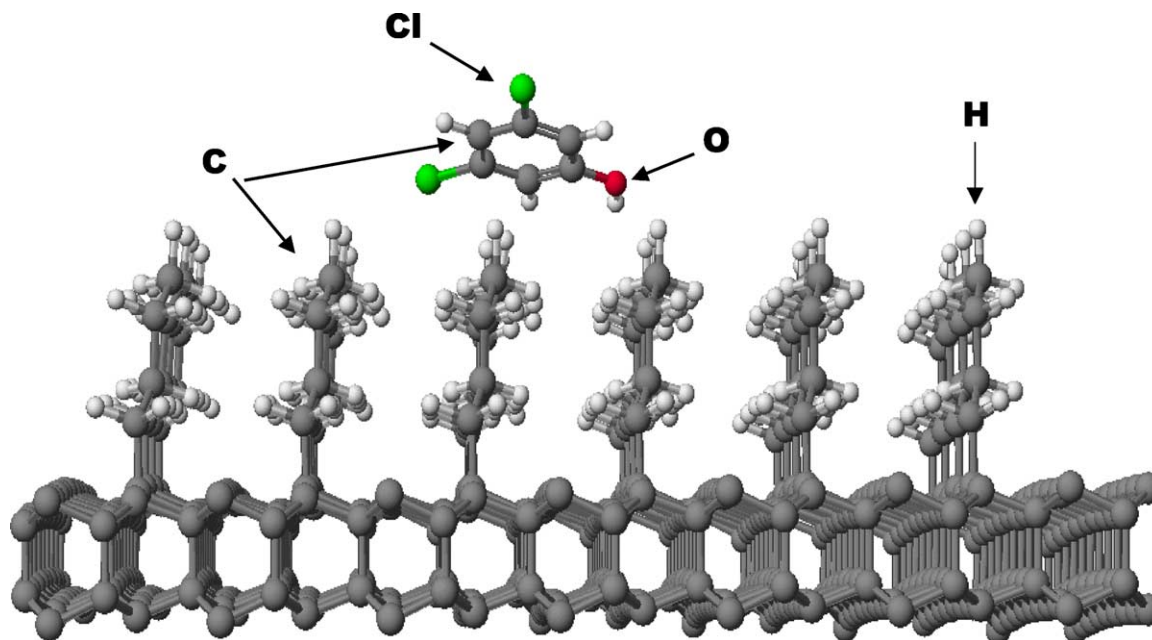


Fig. 1. Optimized structure of adsorption of 3,5-dichlorophenol on the butyl-bonded carbon phase.

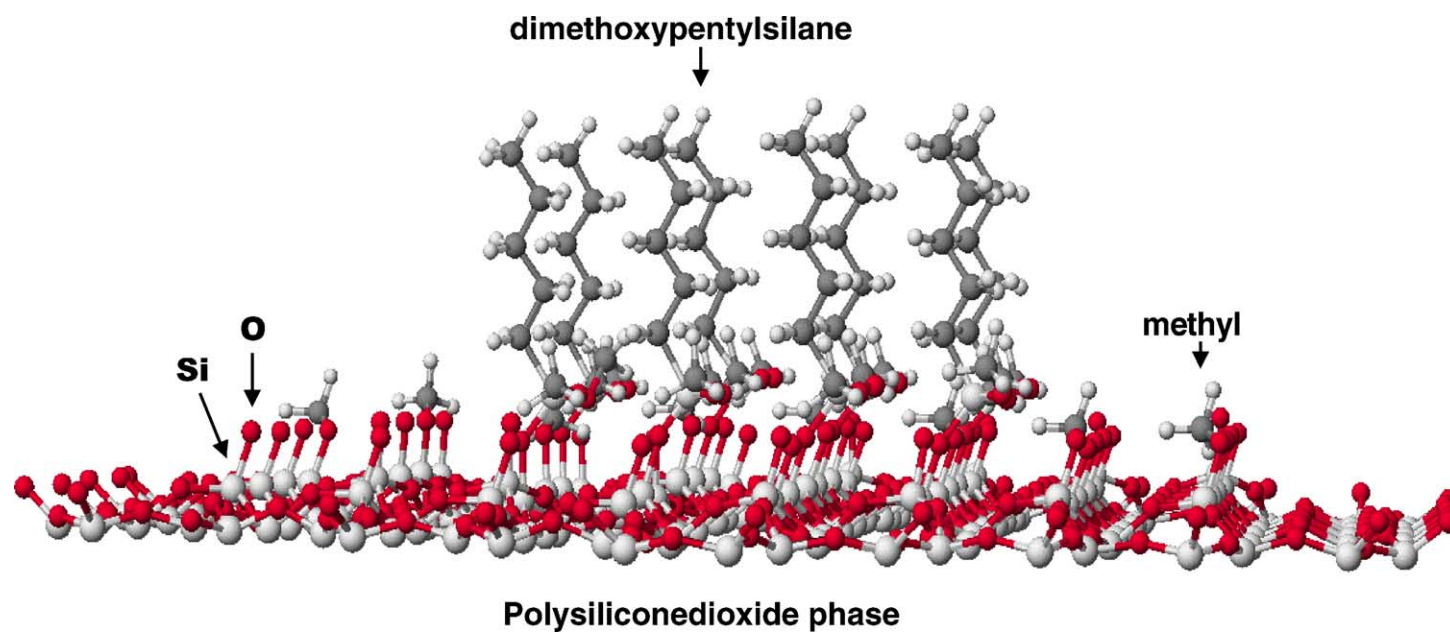


Fig. 2. Dimethoxypentylsilane-bonded polysiliconedioxide phase.

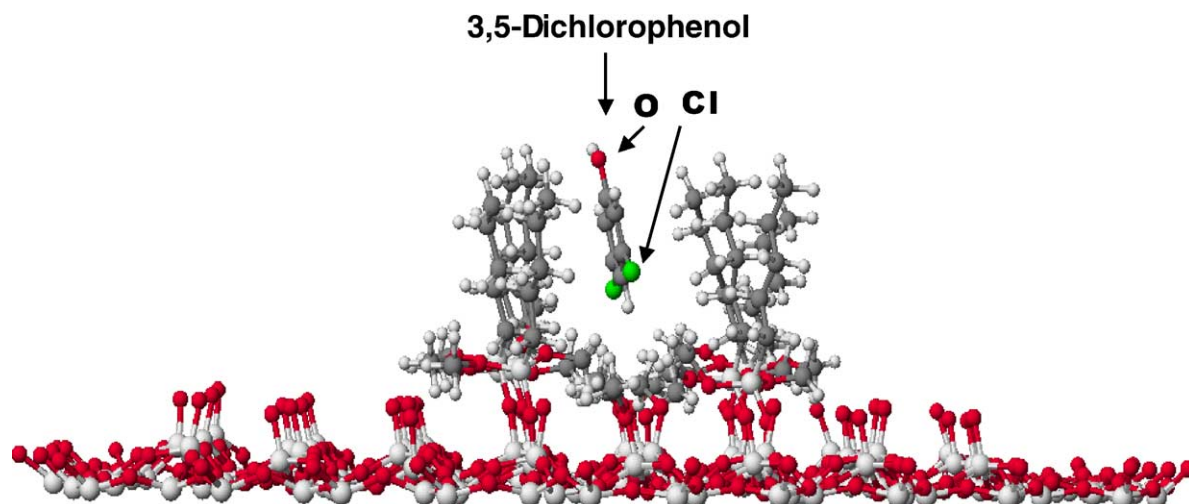


Fig. 3. Optimized structure of adsorption of 3,5-dichlorophenol on the dimethoxyphenylsilane-bonded polysilicondioxide phase.

molecular properties of analytes and model phases and molecular interactions were calculated by molecular mechanics (MM2) from version 5 of the CAChe program from Fujitsu, Tokyo, Japan. The standard parameters used were bond stretch, bond angle, dihedral angle, improper torsion, Van der Waals, hydrogen bond and electrostatic (MM2/MM3 bond dipoles). The Van der Waals cut-off distance was 9 Å. The energy unit was kcal/mole (1 kJ/mol = 4.18 kcal/mol). The Cricket-Graph program from Computer Associates (San Diego, CA, USA) was used for data handling.

### 3. Results and discussion

A model butyl-bonded phase was constructed with highly dense butyl-groups without silanol-group based on the chromatographic performance of pentyl-bonded silica gel [21]. The butyl-bonded phase was a modified carbon phase, and consisted of 628 carbons and 216 hydrogens and 1197 bonds and 6768 connectors. The molecular mass was 7752. The optimized energy value change was less than 0.00001 kcal/mol [17,18]. The molecular size and alkyl-chain length were decided by the calculation capacity of the computer used and the alkyl-chain length effect for the hydrophobicity [22]. The adsorption form of 3,5-dichlorophenol on the butyl-phase is shown in Fig. 1. Improved lap-top computer hardware permitted the construction of a better model bonded-phase. A silica gel based pentyl-bonded phase consisted of 686 atoms, 746 bonds, and 5130 connectors, containing 158 silicones, 304 oxygens, 64 carbons and 160 hydrogens. The mono-layer of the polysilicondioxide phase was locked to avoid deformation of the structure by the further optimization process, because silica gel does not change the atomic distance under liquid chromatographic conditions. The minimized model bonded-phase was constructed by a simple lap-top computer calculation. The structure of the model bonded-phase consists of eight pentyl groups and many oxy-

gens that are kept free to reduce the number of atoms. As shown in Fig. 2, a side view of an optimized structure with 3,5-dichlorophenol is shown in Fig. 3.

The calculated energy values of individual compounds, these two phases and their complexes by MM2 are listed in Table 1 along with the properties,  $\log P$  and  $pK_a$ , of phenolic compounds used. The calculated energy values are final (FS), hydrogen bonding (HB), electrostatic (ES) and Van der Waals (VW) energy values, but HB and ES energy values are not listed due to lack of meaningful contribution to molecular interaction. The details are explained later. The energy values of individual complexes between a model butyl-phase and a phenolic compound are also listed in Table 1. FS1 and VW1 are the final and Van der Waals energy values of a complex between the butyl-bonded phase and a phenolic compound, and FS2 and VW2 are the final and Van der Waals energy values of a complex between the pentyl-bonded phase and a phenolic compound, respectively.

The interaction energy values between a molecular form compound and the model butyl-phase were calculated using MM2 to analyze the retention of molecular form analytes qualitatively:

interaction energy values ( $\Delta$ value)

$$\begin{aligned}
 &= \text{energy value of individual molecule} \\
 &+ \text{energy value of a model phase} \\
 &- \text{energy value of a complex.}
 \end{aligned}$$

The  $r^2$  between  $\Delta$ FS1 or  $\Delta$ VW1 calculated using the model butyl-phase and measured  $\log k$  values of molecular form phenolic compounds listed as pH 4.01/mes in Table 2 was 0.839 ( $n = 38$ ). The retention times were measured by reversed-phase liquid chromatography using an octadecyl-bonded silica gel column, Luna C<sub>18</sub> from Phenomenex (Torrance, CA, USA) at 40 °C in 70% aqueous acetonitrile containing 20 mM sodium phosphate solution,

Table 1  
Molecular properties of phenolic compounds

No.	Chemicals	log <i>P</i>	p <i>K</i> <sub>a</sub>	FS	VW	FS1/ complex	VW1/ complex	FS2/ complex	VW2/ complex
1	1,2-Dihydroxybenzene	0.712	11.5210	-12.3175	2.979	3353.7896	415.946	-639.3091	-338.244
2	1,3-Dihydroxybenzene	0.719	9.3306	-12.4093	2.905	3353.6866	415.878	-639.4068	-338.269
3	1,4-Dihydroxybenzene	0.724	10.4665	-12.3849	2.917	3353.5691	415.748	-639.5571	-338.271
4	1-Hydroxy-2,4-dinitronaphthalene	2.760	-	-17.3047	11.178	3346.5489	421.456	-649.3120	-335.642
5	1-Hydroxynaphthalene	2.720	9.6957	-19.4351	6.132	3344.7302	416.936	-649.3120	-338.254
6	Pentachlorophenol	4.658	6.3080	1.4528	8.713	3363.4253	416.874	-633.3094	-340.415
7	Pentamethylphenol	3.487	10.5879	-6.4902	8.204	3355.9886	417.354	-640.0792	-340.368
8	2,3,4,5-Tetrachlorophenol	4.098	7.2814	-0.9436	7.000	3362.6937	416.533	-633.5105	-340.967
9	2,3,4,5-Tetramethylphenol	3.152	10.4865	-5.8937	7.093	-	-	-640.6586	-341.484
10	2,3,4,6-Tetrachlorophenol	-	-	-4.5807	6.101	3358.2550	415.411	-638.1964	-341.884
11	2,3,4-Trichlorophenol	3.493	7.8493	-2.9751	5.600	3360.9748	416.369	-634.4629	-340.093
12	2,3,4-Trimethylphenol	2.738	10.8922	-9.6638	4.812	3354.2541	415.433	-640.6401	-340.443
13	2,3,5,6-Tetrachlorophenol	4.164	6.5920	-1.5860	5.891	3360.7637	415.021	-633.8383	-341.170
14	2,3,5,6-Tetramethylphenol	3.173	10.3851	-9.8685	5.538	3352.6168	414.419	-643.5258	-342.912
15	2,3,5-Trichlorophenol	3.574	7.5653	-6.1418	4.615	3357.7889	415.297	-638.8230	-342.382
16	2,3,5-Trimethylphenol	2.874	10.7705	-11.1410	3.867	3352.1748	413.854	-642.8024	-342.476
17	2,3,6-Trichlorophenol	3.538	7.0382	-3.3925	4.860	3360.3884	415.447	-635.6925	-341.571
18	2,3,6-Trimethylphenol	2.814	10.4256	-10.5167	4.761	3352.7224	414.680	-642.1185	-340.934
19	2,3-Dibromophenol	-	-	-4.0723	5.077	-	-	-635.7717	-340.638
20	2,3-Dichlorophenol	2.870	8.1130	-4.3435	4.396	3360.7247	416.145	-634.4212	-339.799
21	2,3-Dimethylphenol	2.360	10.2431	-9.5418	4.359	3355.4153	416.065	-639.5455	-339.433
22	2,4,5-Trichlorophenol	3.580	7.5451	-6.1987	4.519	3357.3741	414.841	-638.4696	-342.443
23	2,4,6-Trichlorophenol	3.632	7.0991	-12.6817	4.108	3350.7130	414.148	-645.3557	-342.930
24	2,4,6-Trimethylphenol	2.897	10.5879	-11.9591	4.001	3350.9811	413.566	-643.7867	-341.879
25	2,4-Dibromophenol	3.339	7.8696	-9.7825	3.857	3359.2295	415.069	-641.6017	-342.524
26	2,4-Dichlorophenol	2.971	8.1739	-10.4377	3.653	3354.3640	415.106	-640.5907	-340.708
27	2,4-Dimethylphenol	2.474	10.2837	-10.9887	3.661	3353.6989	415.139	-640.6324	-340.211
28	2,4-Dinitrophenol	1.782	-	-13.3234	5.548	3350.8033	416.572	-642.2304	-337.322
29	2,5-Dichlorophenol	2.955	8.0522	-7.2850	3.629	3357.3709	415.084	-638.1759	-341.466
30	2,5-Dimethylphenol	2.482	10.2025	-10.8232	3.638	3353.4991	414.761	-641.6203	-341.491
31	2,5-Dinitrophenol	1.779	-	-13.2561	5.472	3351.1095	416.662	-643.4423	-339.251
32	2,6-Dibromophenol	3.432	6.8556	-7.2816	4.182	3356.1364	414.368	-638.3781	-340.984
33	2,6-Dichlorophenol	2.921	7.5047	-7.7317	3.876	3357.1179	415.568	-638.1091	-341.006
34	2,6-Dimethylphenol	2.410	10.4256	-11.1215	4.018	3353.0000	414.949	-641.5880	-341.085
35	2,6-Dinitrophenol	1.675	-	-12.8426	6.332	3351.9908	418.011	-642.9613	-337.998
36	2-Bromophenol	2.491	8.3970	-8.0640	3.578	3357.1555	415.599	-636.5530	-339.413
37	2-Chloro-6-methylphenol	2.696	-	-6.7884	4.152	3357.4163	415.189	-637.3251	-340.917
38	2-Chlorophenol	2.225	8.5998	-8.0500	3.432	3357.6962	416.043	-636.2836	-338.997
39	2-Ethylphenol	2.470	10.0605	-9.4427	3.928	3355.1076	415.160	-639.7829	-340.659
40	2-Methylphenol	1.994	10.2025	-10.3036	3.642	3355.2249	415.952	-638.8017	-339.075
41	2-Nitrophenol	1.665	-	-10.5406	4.624	3355.1995	417.176	-638.6554	-337.795
42	2-Hydroxyacetophenone	0.803	9.0263	-3.6999	5.155	3360.6036	416.093	-632.2194	-336.846
43	2-Hydroxynaphthalene	2.749	9.9999	-20.8860	5.702	3342.7851	416.056	-648.7683	-336.211
44	3,4,5-Trichlorophenol	3.569	8.3366	-5.0902	5.228	3359.1339	416.249	-635.5660	-339.580
45	3,4-Dichlorophenol	2.953	8.8235	-6.4075	4.037	3357.6559	414.826	-636.7197	-340.454
46	3,4-Dimethylphenol	2.437	10.3245	-10.5498	3.416	3354.4723	415.203	-639.8363	-340.513
47	3,4-Dinitrophenol	1.754	-	24.8735	4.245	3389.5742	415.681	-603.6873	-338.191
48	3,5-Dichlorophenol	3.033	8.6815	-12.4676	3.392	3352.0456	414.631	-643.7295	-342.232
49	3,5-Dimethylphenol	2.516	10.2636	-11.3779	2.972	3353.6386	414.748	-641.8059	-341.724
50	3-Bromophenol	2.590	9.3711	-9.9042	3.255	3355.2828	415.260	-639.7106	-340.539
51	3-Chlorophenol	2.318	9.2697	-10.0510	3.172	3355.2945	415.332	-638.8110	-339.957
52	3-Ethylphenol	2.536	10.4056	-8.9201	3.985	3354.3640	414.357	-639.6519	-341.193
53	3-Methylphenol	2.059	10.0608	-10.7121	2.977	3354.4499	414.947	-639.2601	-339.763
54	3-Nitrophenol	1.731	-	-10.6460	4.103	3354.6275	416.175	-638.4505	-338.036
55	4-Bromophenol	2.597	9.3711	-9.8803	3.233	3354.6512	414.505	-639.8387	-340.814
56	4-Chloro-2-methylphenol	2.732	-	-9.3482	3.834	3355.2375	415.486	-640.2602	-340.885
57	4-Chloro-3,5-dimethylphenol	3.083	-	-10.7196	4.522	3353.1956	415.333	-642.3404	-341.562
58	4-Chloro-3-methylphenol	2.698	-	-10.3893	3.753	-	-	-639.9979	-340.110
59	4-Chlorophenol	2.316	9.5334	-10.0243	3.155	3355.2130	415.194	-639.9198	-339.964
60	4-Ethylphenol	2.523	10.3853	-8.9664	3.992	3353.4848	413.618	-639.5023	-340.608

Table 1 (Continued)

No.	Chemicals	log <i>P</i>	p <i>K</i> <sub>a</sub>	FS	VW	FS1/ complex	VW1/ complex	FS2/ complex	VW2/ complex
61	4-Methylphenol	2.060	10.1216	−10.7544	2.975	3354.5361	415.084	−639.4583	−339.949
62	4-Nitrophenol	1.751	–	−10.7284	4.091	3354.8881	416.463	−638.9058	−338.709
63	4-Hydroxybutylbenzoate	1.114	8.9249	−4.8077	8.376	3354.1263	414.192	−642.4034	−344.414
64	4-Hydroxypropylbenzoate	0.574	8.9249	−5.4543	7.925	3355.4685	415.344	−641.5860	−343.001
65	4- <i>tert</i> -Butylphenol	3.180	10.2839	−6.3765	5.425	3355.7475	414.300	−638.7314	−339.952
66	Phenol	1.574	10.0405	−10.2105	2.960	3356.0700	416.126	−637.0901	−337.751
	Butyl-bonded phase	–	–	3373.0355	419.960	–	–	–	–
	Pentyl-bonded phase	–	–	−612.9215	−327.343	–	–	–	–

log *P* (*N*log *P*) and p*K*<sub>a</sub> are from references 9,10, FS and VW are energy values of final structure and van der Waals (kcal/mol), FS1/complex and VW1/complex are energy values (kcal/mol) of complexes with butyl-bonded phase, and FS2/complex and VW2/complex are energy values (kcal/mol) of complexes with pentyl-bonded phase.

Table 2

Measured and predicted retention factors of phenolic compounds

No.	Chemicals	Retention factors					
		pH 4.01/mes	<i>k</i> <sub>max</sub> /log <i>P</i>	<i>k</i> <sub>max</sub> /MI	pH 8.49/mes	pH 8.49/log <i>P</i>	pH 8.49/MI
1	1,2-Dihydroxybenzene	–	0.345	0.637	–	0.345	0.636
2	1,3-Dihydroxybenzene	0.377	0.347	0.637	0.360	0.303	0.556
3	1,4-Dihydroxybenzene	0.261	0.348	0.659	0.280	0.344	0.652
4	1-Hydroxy-2,4-dinitronaphthalene	–	1.262	1.820	–	–	–
5	1-Hydroxynaphthalene	1.181	1.230	1.119	1.159	1.155	1.054
6	Pentachlorophenol	3.132	4.198	2.911	0.084	0.027	0.019
7	Pentamethylphenol	–	2.000	2.317	–	1.984	2.299
8	2,3,4,5-Tetrachlorophenol	–	2.944	2.280	–	0.172	0.133
9	2,3,4,5-Tetramethylphenol	–	–	2.917	–	1.602	2.888
10	2,3,4,6-Tetrachlorophenol	–	1.618	2.328	–	–	–
11	2,3,4-Trichlorophenol	1.788	2.009	1.535	0.140	0.374	0.286
12	2,3,4-Trimethylphenol	–	1.245	1.390	–	1.240	1.384
13	2,3,5,6-Tetrachlorophenol	–	3.076	1.782	–	0.038	0.022
14	2,3,5,6-Tetramethylphenol	–	1.641	2.350	–	1.620	2.320
15	2,3,5-Trichlorophenol	2.099	2.113	1.941	0.095	0.225	0.206
16	2,3,5-Trimethylphenol	1.494	1.358	1.589	1.510	1.351	1.580
17	2,3,6-Trichlorophenol	1.800	2.065	1.799	0.042	0.070	0.061
18	2,3,6-Trimethylphenol	1.636	1.306	1.570	1.648	1.291	1.552
19	2,3-Dibromophenol	–	–	1.600	–	–	–
20	2,3-Dichlorophenol	1.220	1.355	1.164	0.310	0.401	0.344
21	2,3-Dimethylphenol	1.138	0.979	1.148	1.151	0.962	1.128
22	2,4,5-Trichlorophenol	2.009	2.123	1.791	0.110	0.216	0.183
23	2,4,6-Trichlorophenol	2.131	2.193	1.936	0.051	0.086	0.076
24	2,4,6-Trimethylphenol	1.696	1.377	1.641	1.710	1.366	1.628
25	2,4-Dibromophenol	1.657	1.824	1.637	0.650	0.320	0.316
26	2,4-Dichlorophenol	1.282	1.442	1.183	0.468	0.470	0.385
27	2,4-Dimethylphenol	1.173	1.054	1.069	1.180	1.037	1.052
28	2,4-Dinitrophenol	–	0.679	0.927	–	–	–
29	2,5-Dichlorophenol	1.326	1.429	1.365	0.390	0.382	0.365
30	2,5-Dimethylphenol	1.167	1.059	1.340	1.169	1.039	1.314
31	2,5-Dinitrophenol	0.832	0.678	1.189	0.029	–	–
32	2,6-Dibromophenol	1.588	1.932	1.422	0.086	0.044	0.032
33	2,6-Dichlorophenol	1.282	1.400	1.236	0.091	0.131	0.116
34	2,6-Dimethylphenol	1.274	1.012	1.256	1.279	1.000	1.242
35	2,6-Dinitrophenol	0.583	0.635	1.175	0.031	–	–
36	2-Bromophenol	0.964	1.064	0.853	0.750	0.475	0.381
37	2-Chloro-6-methylphenol	1.173	1.213	1.274	1.009	–	–
38	2-Chlorophenol	0.890	0.899	0.813	0.700	0.506	0.458
39	2-Ethylphenol	1.231	1.052	1.227	1.239	1.025	1.195
40	2-Methylphenol	0.901	0.778	0.855	0.910	0.763	0.839
41	2-Nitrophenol	1.148	0.631	0.793	0.110	–	–
42	2-Hydroxyacetophenone	–	0.366	0.859	–	0.283	0.665

Table 2 (Continued)

No.	Chemicals	Retention factors					
		pH 4.01/mes	$k_{\max}/\log P$	$k_{\max}/\text{MI}$	pH 8.49/mes	pH 8.49/ $\log P$	pH 8.49/MI
43	2-Hydroxynaphthalene	1.043	1.253	0.759	1.030	1.215	0.736
44	3,4,5-Trichlorophenol	–	2.109	1.259	–	0.870	0.519
45	3,4-Dichlorophenol	1.335	1.426	1.219	0.920	0.974	0.833
46	3,4-Dimethylphenol	1.005	1.028	0.998	1.021	1.013	0.983
47	3,4-Dinitrophenol	–	0.668	0.865	–	–	–
48	3,5-Dichlorophenol	1.643	1.500	1.469	0.680	0.913	0.894
49	3,5-Dimethylphenol	1.065	1.081	1.247	1.074	1.063	1.227
50	3-Bromophenol	1.049	1.135	1.104	0.940	1.003	0.976
51	3-Chlorophenol	0.965	0.955	0.899	0.880	0.819	0.771
52	3-Ethylphenol	1.082	1.096	1.324	1.074	1.083	1.308
53	3-Methylphenol	0.823	0.809	0.863	0.830	0.788	0.840
54	3-Nitrophenol	0.695	0.658	0.746	0.360	–	–
55	4-Bromophenol	1.017	1.134	1.138	0.970	1.006	1.005
56	4-Chloro-2-methylphenol	1.324	1.242	1.371	1.300	–	–
57	4-Chloro-3,5-dimethylphenol	1.594	1.549	1.574	1.560	–	–
58	4-Chloro-3-methylphenol	1.205	1.213	1.059	1.169	–	–
59	4-Chlorophenol	0.925	0.953	0.925	0.890	0.874	0.848
60	4-Ethylphenol	1.091	1.086	1.274	1.099	1.072	1.257
61	4-Methylphenol	0.823	0.811	0.891	0.830	0.792	0.871
62	4-Nitrophenol	0.634	0.667	0.804	0.040	–	–
63	4-Hydroxybutylbenzoate	–	0.445	5.070	–	0.325	3.707
64	4-Hydroxypropylbenzoate	–	0.316	3.811	–	0.231	2.786
65	4- <i>tert</i> -Butylphenol	1.730	1.730	1.820	1.734	1.703	1.791
66	Phenol	0.649	0.596	0.622	0.661	0.580	0.605

Retention factors measured at pH 4.01 (pH 4.01/mes) and 8.49 (pH 8.49/mes) and predicted from  $N\log P$  (pH 4.01/ $\log P$ ) and (pH 8.49/ $\log P$ ) are from references [12] and [24]. Retention factors (pH 4.01/MI) and (pH 8.49/MI) were predicted from molecular interactions (MI).

pH 2–10 [20]:

$$\Delta\text{FS1} = 6.072 (\log k_{\max}) + 8.015, \\ r^2 = 0.839, n = 38 \quad (1)$$

$$\Delta\text{VW1} = 6.619 (\log k_{\max}) + 8.127, \\ r^2 = 0.839, n = 38 \quad (2)$$

The outliers were nitro-substituted phenols, dihydroxybenzenes and *tert*-butyl and ethyl-phenols. The retention time of dihydroxybenzenes were too short. In this model system, one side of the analyte contacted with this model phase, and the steric effect may not be neglected for *tert*-butylphenol and ethylphenols.

An improvement in the correlation was expected if a low density phase was used as a model phase because an analyte should be buried in the alkyl chains. The interaction energy values between a phenolic compound and the silica gel based pentyl-phase were calculated. The  $r^2$  between  $\Delta\text{FS2}$  calculated using the model pentyl-phase and measured  $\log k$  values of molecular form phenolic compounds listed as pH 4.01/mes in Table 2 improved to 0.914 ( $n = 42$ ), and all alkyl-substituted phenols were included in the calculation. The correlation ( $r^2$ ) was 0.890 ( $n = 42$ ) from  $\Delta\text{VW2}$  energy values. The outliers are nitro-substituted phenols and dihydroxybenzenes. The poor results for nitro-substituted phenols may be due to the difficulty of computational

chemical calculation of nitro-group that observed for  $\log P$  prediction:

$$\Delta\text{FS2} = 10.738 (\log k_{\max}) + 16.453, \\ r^2 = 0.914, n = 42 \quad (3)$$

$$\Delta\text{VW2} = 10.893 (\log k_{\max}) + 16.257, \\ r^2 = 0.890, n = 42 \quad (4)$$

The contribution of HB2 and ES2 values not listed in Table 1 can be neglected due to the very poor  $r^2$  values at moment. The HB2 energy value of these model phase is zero. The  $r^2$  for  $\Delta\text{HB2}$  and  $\Delta\text{ES2}$  was 0.034 and 0.311, respectively. The contribution of  $\Delta\text{VW}$  energy indicated that hydrophobic interaction is the predominant molecular interaction in the retention of these phenolic compounds on an alkyl-bonded phase in reversed-phase liquid chromatography.

The retention factors of their partially ionized compounds were calculated by Eq. (5) [23] using  $\text{p}K_a$  values predicted from the partial charge of the hydrogen of the phenolic hydroxy-group [12]:

$$k = \frac{k_m + k_i(K_a/[\text{H}^+])}{1 + (K_a/[\text{H}^+])} \quad (5)$$

where,  $k_m$  and  $k_i$  are the retention factors of the molecular and ionized analytes, respectively, and  $K_a$  is the dissociation constant of analytes. The  $\text{H}^+$  is hydrogen ion concentration in eluent. The correlation between the retention factors

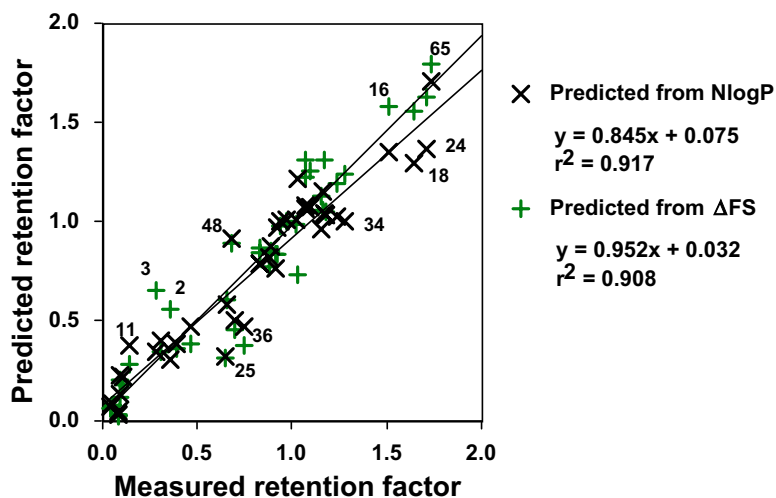


Fig. 4. Relation between predicted and measured retention factors at pH 8.49. Numbers beside symbols see Table 2.

measured and predicted by the former method using  $N \log P$  was obtained from the Eq. (6) at pH 8.49, and that by this new method using molecular interaction energy,  $\Delta FS2$ , was obtained from Eq. (7) at pH 8.49. The measured and predicted retention factors of phenolic compounds are given in Table 2:

$$y = 0.845x + 0.075, \\ r^2 = 0.917, n = 41 \quad (\text{from } N \log P) \quad (6)$$

$$y = 0.952v + 0.032, \\ r^2 = 0.908, n = 41 \quad (\text{from } \Delta FS2) \quad (7)$$

The above results indicated that the retention time of phenolic compounds can be predicted using both energy value changes in the optimized structure calculated by MM2 and  $\log P$  values calculated by MOPAC. The addition of  $pK_a$  values predicted from the atomic partial charge calculated by MOPAC enables the retention factors in a given pH eluent to be predicted. The relations in Eqs. (6) and (7) are shown in Fig. 4.

Molecular interaction in liquid chromatography can be quantitatively estimated from the energy values calculated by molecular mechanics using analytes and a model phase. The addition of a solvation effect and the construction of a better model phase should improve the precision of qualitative analysis of retention factors in liquid chromatography.

#### 4. Conclusion

The retention time of phenolic compounds in reversed-phase liquid chromatography was predicted by molecular interaction energy values calculated using MM2. The precision of predicted the retention factors by this new method was equivalent to a former method in which the retention time was predicted by  $\log P$  calculated using MOPAC.

Furthermore, the prediction of retention factors of phenolic compounds in reversed-phase liquid chromatography in a given pH eluent was performed using the dissociation constant ( $pK_a$ ) from the atomic partial charge calculated using MOPAC. Further computational chemical study with a solvent effect using a better model-phase will improve the precision.

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