Estimation for Retention Factor of Isoflavones in Physico-Chemical Properties

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The estimation of retention factors by correlation equations with physico-chmical properties maybe helpful in chromatographic work. The physico-chemical properties were water solubility (S), hydrophobicity (P), total energy (E_t), connectivity index 1 ($^1\chi$), hydrophilic-lipophlic balance (x) and hydrophilic surface area (h) of isoflavones. The retention factors were experimentally measured by RP-HPLC. Especially, the empirical regulations of water solubility and hydrophobicity were expressed in a linear form. The equation between retention factors and various physico-chemical properties of isoflavones was suggested as $k = a_0 - a_1 \log S + a_2 \log P^{a_2} + a_3(E_t) + a_4(^1\chi) - a_5(x) - a_6(h)$, and the correlation coefficients estimated were relatively higher than 0.95. The empirical equations might be successfully used for a prediction of the various chromatographic characteristics of substances, with a similar chemical structure.

Key Words : Physico-chemical properties, Retention factors, Correlation relationships

Introduction

Recently, there has been an increasing tendency to apply reversed-phase high-performance liquid chromatography (RP-HPLC) in studies of quantitative structure-property/activity relationships (QSPAR, this abbreviation is used frequently for a designation of such equations).[1,2] The research of interrelation between the chromatographic characteristics and the physico-chemical properties of biologically active substances becomes the important task in modern chemistry.

Isoflavones are important biologically active substances contained in a variety of plants mainly including soybeans. Especially, phytoestrogens have a high potential in the prevention of atherosclerosis, osteoporosis, cardiovascular diseases, several kinds of the cancer and postmenopausal complaints.[3-5] The important group of phytoestrogens in isoflavones includes daidzein, glycitein and genistein. And each of the three isoflavones of daidzein, glycitein and genistein has the four different isomers forms in soy food products. In nature, the 6"-*O*-malonyl-*β*-glucosides of the isoflavones are the predominant forms. The 6"-*O*-acetyl-*β*-glucoside-, *β*-glucoside-, and aglycone forms develop from the 6"-*O*-malonyl-*β*-glucosides during processing of soybeans and soy foods or during sample preparation and analysis.[6,7] There were few studies on the prediction of physico-chemical properties for organic compounds by the linear logarithm relationship between the retention factors and physico-properties of the targeted compounds.[8] Thus, physico-properties[9] and structure properties[10] have a several kinds of descriptors and properties, which are, for example, refractive index, ion mobility of organic compound, partition coefficient, aqueous solubility of liquids and solids and wiener index, and so on. In this study, the retention of isoflavones are assumed to be governed by their physico-chemical properties and structure properties such as water solubility (S), hydrophobicity (P), total energy (E_t), connectivity index ($^1\chi$), hydrophilic-lipophilic balance (x) and hydropilic surface area (h). Especially, the water solubility and hydrophobicity are the most available and precisely measured data for the investigated molecules. In this work, 12 isoflavones are divided into three Groups; daidzein, 6"-*O*-malonyl-*β*-glucoside-daidzin, 6"-*O*-acetyl-*β*-glucoside-daidzin, and *β*-glucoside-daidzin (Group 1), glycitein, 6"-*O*-malonyl-*β*-glucoside-glycitin, 6"-*O*-acetyl-*β*-glucoside-glycitin, and *β*-glucoside-glycitin (Group 2), and finally genistein, 6"-*O*-malonyl-*β*-glucoside-genistin, 6"-*O*-acetyl-*β*-glucoside-genistin, and *β*-glucoside-genistin (Group 3).

The search of the correlation relationships between physico-chemical properties and the chromatographic retention factor of isoflavones was the purpose of this work. The correlations were established by the empirical equations. The retention factors of isoflavones were demonstrated to confirm the feasibility of the correlations.

Theoretical Background

The retention factor (k) was defined as $k = (t_R - t_m)/t_m$, where t_R is the retention time of the compound, and t_m, the dead time. The calculations of physico-chemical properties were performed using the ChemSW[11] and HyperChem program.[12,13] The total energy (E_t) of all the isoflavones have been obtained by a semi-empirical method AM1 in the package HyperChem. The values of water solubility (S), hydrophobicity (P), connectivity index 1 ($^1\chi$), hydrophilic-lipophlic balance (x) and hydrophilic surface area (h) were obtained by the ChemSW.

Hydrophobicity. The logarithm of the partition coefficient between *n*-octanol and water was sometimes referred to as $\log K_{ow}$. This value is assumed to be a good indicator of the relative lipophilicity of a molecule. In ChemSW, the four method of fragment addition generally following the methods

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of Hansch and Leo,[14,15] atom based generally following Ghose and Crippen,[16] charge and atom based, and Q Log P[17] were available, but in this work the value of Q Log P was used for better prediction.

Connectivity index. The connectivity index was calculated using the bond matrix. Each nonhydrogen atom i was characterized by the value L_i, which corresponds to the number of atoms attached to it, except for hydrogen atoms; a π bond was taken into account by adding nuity to the L_i value. Then the P_i values, which is equal to the product of L_i for two neighboring atoms were calculated and square root (Ci) from each P_i value was extracted. The sum of all Ci provides the topological connectivity index 1.[18,19]

HLB (hydrophilic-lipophilic balance). For nonionic molecules the minimum value is 0 and the maximum value is 20. This scale was invented by Griffin as a measure of the emulsifying ability of surfactants.[20] Anionic surfactant molecules often have values above 20. Emulsifying agents in water usually have an HLB between 5 and 14. Emulsion drop size usually declines as HLB rises.

Results and Discussion

In this work, the retention factors for organic compounds were estimated by the mathematical relationships expressed as a linear relationship in terms of physico-chemical proper-

Table 1. Retention factors and physico-chemical properties of isoflavones

No.	Groups	Property / Compound	k [−]	log S [mol/M^3 water]	log P^Q [−]	E_t [kcal/mol]	$^1\chi$ [−]	x [−]	h [cm^2/mol × 10^9]
1	1	Daidzin	4.707	2.8553	1.3076	135.509	14.384	13.088	16.179
2	2	Glycitin	5.161	2.7594	1.3341	146.443	15.333	12.312	16.595
3	3	Genistin	8.697	3.8221	0.8470	142.904	14.795	14.880	18.715
4	1	Malonyl daidzin	9.151	1.9749	1.7464	167.177	17.134	14.519	21.391
5	2	Malonyl glycitin	9.606	1.8790	1.7730	178.104	18.083	13.773	21.806
6	1	Acetyl daidzin	12.586	1.7849	2.3648	149.436	15.740	12.261	23.927
7	2	Acetyl glycitin	13.293	1.6890	2.3914	160.368	16.689	11.616	16.996
8	3	Malonyl genistin	13.798	2.9417	1.2858	178.104	17.545	15.980	17.412
9	1	Daidzein	16.626	0.7403	3.2794	77.638	9.148	5.780	19.532
10	2	Glycitein	17.687	0.6444	3.3059	88.608	10.097	6.987	4.860
11	3	Acetyl genistin	18.192	2.7517	1.9043	156.831	16.151	13.895	6.454
12	3	Genistein	22.737	1.7071	2.8188	85.033	9.558	13.088	8.575

Table 2. Empirical equations of various physico-chemical properties with groups

Groups	Equations		r^2	
1	$k = -5.7107 \log S + 21.269$ $k = 5.824 \log P^Q - 1.8969$		0.9581 0.9658	
	with β-glucosides	without β-glucosides	w	w/o
	$k = -8 \times 10^{-5} E_t + 21.488$ $k = -0.8947\ ^1\chi + 23.385$ $k = -1.0386\ x - 22.62$ $k = -0.4918\ h - 18.074$	$k = -8 \times 10^{-5} E_t + 22.73$ $k = -0.8346\ ^1\chi - 24.478$ $k = -0.8040\ x - 21.514$ $k = -0.4267\ h - 18.939$	0.3837 0.3796 0.6292 0.4680	0.9190 0.9054 0.9501 0.9535
2	$k = -6.0112 \log S + 21.914$ $k = 6.1515 \log P^Q - 2.103$		0.9586 0.9730	
	with β-glucosides	withoutβ-glucosides	w	w/o
	$k = -9 \times 10^{-5} E_t + 23.967$ $k = -0.9656\ ^1\chi - 25.97$ $k = -1.0386\ x - 22.62$ $k = -0.5648$ h + 20.228	$k = -8 \times 10^{-5} E_t + 25.192$ $k = -0.9035\ ^1\chi - 27.041$ $k = -1.1527\ x - 25.968$ $k = -0.5023\ h - 21.176$	0.4035 0.3993 0.6292 0.4731	0.921 0.9075 0.9756 0.9638
3	$k = -6.7402 \log S - 34.767$ $k = 6.9053 \log P^Q + 4.0205$		0.9487 0.9650	
	with β-glucosides	without β-glucosides	w	w/o
	$k = -9 \times 10^{-5} E_t + 28.801$ $k = -1.0555\ ^1\chi - 31.174$ $k = -2.1524\ x - 45.703$ $k = -0.6217\ h - 26.852$	$k = -9 \times 10^{-5} E_t + 30.523$ $k = -0.9844\ ^1\chi + 32.436$ $k = -1.6747\ x - 40.899$ $k = -0.5502\ h - 27.786$	0.3722 0.3755 0.6607 0.4511	0.9161 0.8826 0.9879 0.9471

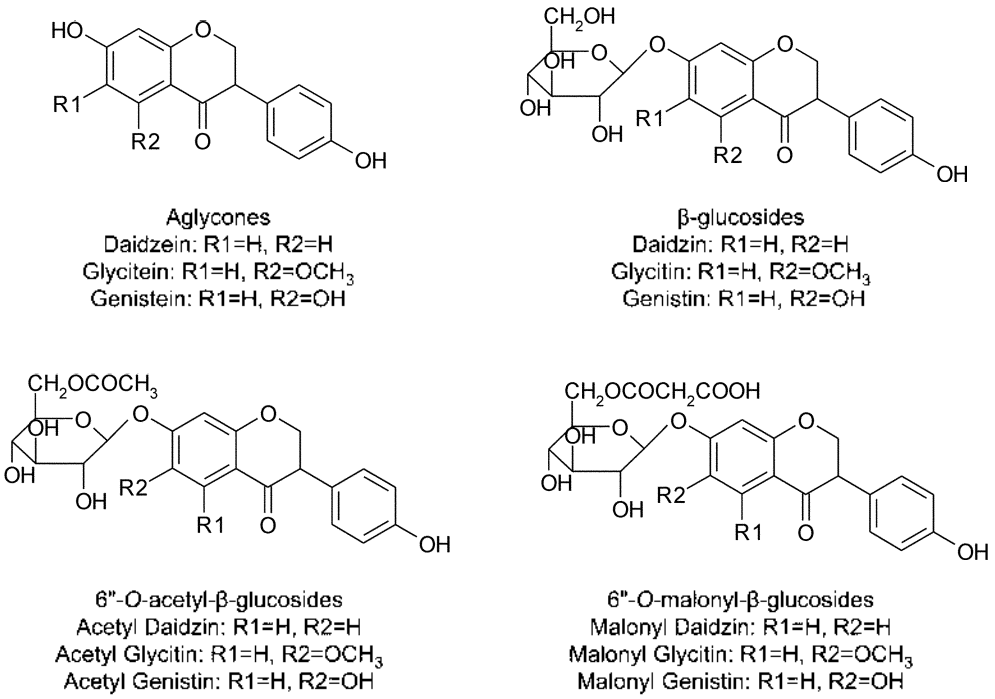

Figure 1. Chemical structures of isoflavones.

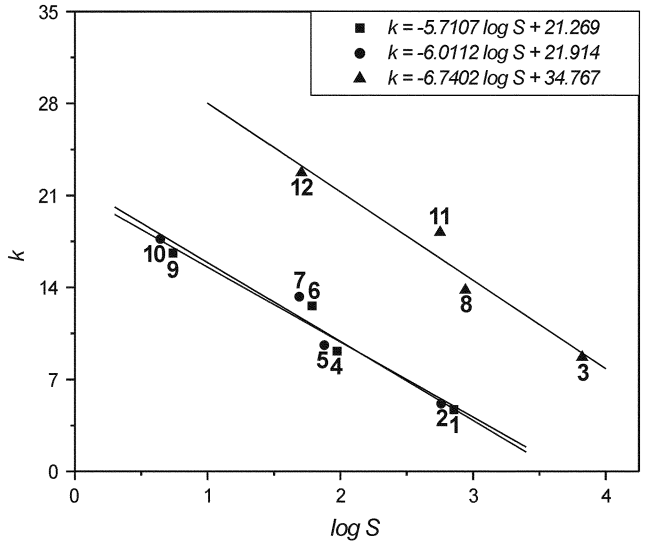

Figure 2. Correlation of k in descriptor of log S with groups (■ : Group 1. ● : Group 2. ▲ : Group 3).

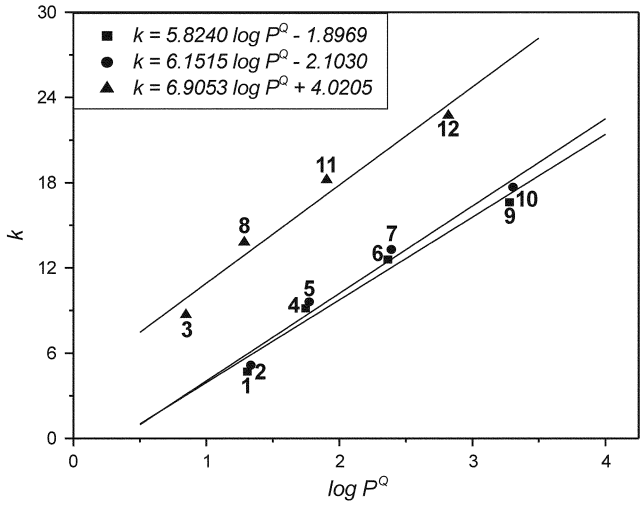

Figure 3. Correlation of k in descriptor of log P^{Q} with groups (■ : Group 1. ● : Group 2. ▲ : Group 3).

ties of the target compounds. First of all, the retention factor (k) was obtained by reversed-phase HPLC.[21] The binary mobile phase of water with 0.1% acetic acid and acetonitrile with 0.1% acetic acid was used. The mobile phase composition of acetonitrile with 0.1% acetic acid was linearly changed from 15 to 35% over 50 min. The flow rate of mobile phase was fixed at 1 mL/min and monitored at the fixed wavelength of 254 nm.

The retention factors and calculated physico-chemical properties of isoflavones were listed in Table 1. And the structures and names of isoflavones are given in Figure 1. To develop a relationship between them, the linear and semi-log functions were tried. By a regression analysis, the resulting correlations were suggested in Table 2. Especially, the semi-log linear equation between the retention factors and the descriptors of water solubility (S) and hydrophobicity (P^{Q}) were satisfactory. The linear empirical equations of Groups 1, 2, and 3 were $k = -5.7107 \log S + 21.269$, $k = -6.0112 \log S + 21.914$ and $k = -6.7402 \log S + 34.767$, respectively. And the correlation coefficients were higher than 0.94. Figure 2 showed the dependence of the retention factor for the isoflavones on the water solubility. The retention factor increased with a descreses in log S. For the log P^{Q} best-fit of

Table 3. Combined retention factor in linear form

$k = a_0 + a_1\log S - a_2\log P^O + a_3(E_t) + a_4(^1\chi) + a_5(x) - a_6(h)$	a_0	a_1	a_2	a_3	a_4	a_5	a_6	r^2
1 group	4.4306	−0.9125	2.7578	1.34×10^{-5}	1.4674	−1.995	0.1534	0.9997
2 group	4.5475	−1.1483	3.4455	-8.21×10^{-5}	2.2473	−1.7932	−0.0611	0.9848
3 group	4.7634	−2.5691	4.9722	-2.02×10^{-5}	1.6284	−0.0412	−0.5906	0.9999

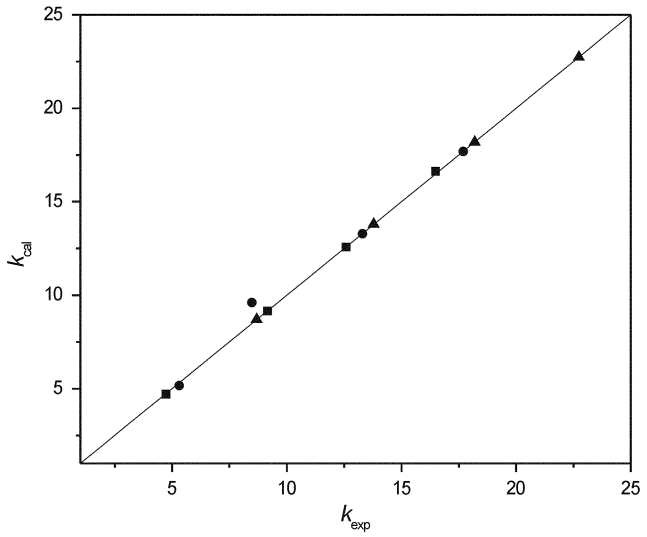

Figure 4. Comparisons of the experimental data and the calculated retention factors by combined equation (■ : Group 1, ● : Group 2, ▲ : Group 3).

empirical equations of Groups 1, 2, and 3 were $k = 5.824 \log P^O - 1.8969$, $k = 6.1515 \log P^O - 2.103$ and $k = 6.9053 \log P^O + 4.0205$, respectively. The relationships between retention factor and hydrophobicity were observed in Figure 3. Contrary to the trend in log *S*, the retention factor increased with log P^O. But the correlation coefficients of the linear equation the parameters of E_t, $^1\chi$, *x* and *h* and the retention factors of isoflavones were significantly lower than those of log *S* and log P^O (Table 2). It resulted from the fact that the presence in β-glucoside residue reduced the retention factor of isoflavones. The substitution of the glucoside residue for the protons of the hydroxyl group in the β-glucoside-daidzin, β-glucoside-glycitin, and β-glucoside-genistin sharply renders the decreased retention due to the strong specific interaction with polar eluent solvent. All natural isoflavones containing glycoside groups in their molecules have small values of *k*.[22] With the parameters of E_t, $^1\chi$, *x* and *h*, the linear equations were estimated with groups 1, 2, and 3 with and without β-glucoside. The regression coefficients without β-glucoside were mostly higher than 0.9, while those with β-glucoside were pretty low, because the isoflavones with β-glucoside were very soluble in water and the good correlations could not be established without considering water solubility.

For the isoflavone compounds, the retention factor was expressed as a linear equation, so all the physico-chemical properties were linearly combined. The multiple-linear equations of the retention factors with various physico-chemical properties of isoflavones were listed in Table 3. The mathematical expression was $k = a_0 + a_1\log S + a_2\log P^O + a_3(E_t) + a_4(^1\chi) + a_5(x) + a_6(h)$, and the correlation coefficients of the Groups 1, 2, and 3 are 0.9694, 0.9754, and 0.9530, respectively. Comparisons of the calculated retention factors in combined equation with the experimental variables were shown in Figure 4. The equations might be successfully utilized for a prediction of the various chromatographic characteristics of substances, which have a similar chemical structure (structural homologies).

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