SMGroup

Annals of Chromatography and Separation Techniques

Research Article

Quantitative *In Silico* Analysis of HILIC Retention Mechanisms

Toshihiko Hanai*

Health Research Foundation, Research Institute for Production Development 4F, Japan

Abstract

A simple chromatographic experiment was performed using theoretically stable and inert bonded-phase silica gels and molecular interaction energy values were calculated using a molecular mechanics calculation to obtain a quantitative explanation of the Hydrophilic Interaction Liquid Chromatography (HILIC) retention mechanisms. We found that the polar groups of the analytes were in contact with the polar groups of bonded phases. The molecular interactions and the removable of the analytes from the bonded-phases depended on the properties of the eluent components used in the chromatographic experiment. The interactions can be quantitatively analyzed from the calculated hydrogen-bonding and electrostatic energy values.

Article Information

Received date: Oct 10, 2017
Accepted date: Oct 20, 2017
Published date: Oct 25, 2017

***Corresponding author**

Toshihiko Hanai, Health Research Foundation, Research Institute for Production Development 4F, Sakyo-ku, Kyoto 606-0805, Japan,
Tel: 81-45-547-4871;
E-mail: thanai@kf7.so-net.ne.jp

Keywords HILIC; Retention mechanism; In silico; Polar phase

Introduction

The retention of analytes on or in a stationary phase depends on the physicochemical interactions between the analytes and the stationary phase material. When a strong solvent, in which the solute readily dissolves, is used for elution, the solute elutes very quickly from the column. The forces holding an analyte on the stationary phase are similar to those responsible for its dissolution in the solvent. Eight solubility factors are recognized: van der Waals (a combination of van der Waals volume, repulsion, and London dispersion) force, dipole-dipole, ion-dipole, Coulombic, and repulsion forces, charge-transfer complexation, hydrogen-bonding and coordination bonds. Molecular interactions are probably responsible for retention in liquid chromatography and can be explained by these solubility factors. However, the retention of a particular molecule is not due to a single factor but rather to a combination of several factors. The probable interactions can be estimated from the chemical structure of the analytes and the stationary phase materials and the chromatographic mode [1]. Practically, the above forces can be classified as Van der Waals force, hydrogen bonding, and electrostatic interaction.

Hydrogen bonding and electrostatic interaction are hydrophilic interactions. In general, hydrophobic interactions are mainly used to explain the mechanism of reversed-phase liquid chromatography. However, HILIC is not the same as normal-phase liquid chromatography. Even the retention mechanisms of normal-phase liquid chromatography involve hydrogen-bonding and weak electrostatic interactions (dipole-dipole, π–π, and charge-transfer interactions). If we eliminate the "hydrophobic interactions" from the retention mechanisms in liquid chromatography, the remaining interactions are hydrogen-bonding and electrostatic interaction. The difference between HILIC and normal-phase liquid chromatography seems to be the properties of the solvents used in the eluent. In general, normal-phase liquid chromatography uses only organic solvents; however, a water-saturated organic solvent is often used to improve the separation. Ion-exchange liquid chromatography is independent of HILIC. Thus, the retention mechanism of HILIC was analyzed.

Several problems regarding the retention mechanism of HILIC mode chromatography have been reported in the literature [2-8] mostly relating to the basic problem of quantitatively studying the stationary phase selectivity. The stability of silica gels and the polar bonded phases hinder the reproducibility of normal-phase liquid chromatography, and the results vary when such columns are aged. In particular, polar bonded silica gels are unstable in aqueous eluents containing buffer components. Moreover, the adsorption of water to the soluble silica matrix under such conditions renders the stationary phase unstable. Furthermore, manufacturers do not provide a long-life guarantee for such bonded-phase silica gels. Therefore, explanations using multi-mode, mixed-mode, and double-mode separation confuse beginners and should be simplified. The possible molecular interactions depend on the properties of the analytes, packing materials, and eluents. In addition, the retention mechanisms proposed in the literature are not quantitatively described like those of other liquid chromatographic retention mechanisms. However, these factors can be classified using the solubility factors such as van der Waals force, hydrogen bonding, electrostatic interaction, and steric hindrance. In ion-exchange liquid chromatography, ions are exchanged based on the strength of the electrostatic force [1]. Charge-transfer-type interactions are based on the localization of electrons in the chromatography process, and this phenomenon is used to teach organic chemistry quantitatively.

Table 1: Properties of analytes.

Chemicals	log *P*	p*Ka*	Hx/C_8*	NH_2/C_8*	Gua/C_8*
Benzoic acid	1.61	4.20	1.25	1.32	1.46
Phenol	1.29	10.02	1.07	1.02	0.76
4-Chlorophenol	1.77	9.38	1.01	0.94	0.63
2,4-Dichlorophenol	2.25	9.23	0.99	0.89	0.55
2,4,6-Trichlorophenol	2.80	9.39	0.99	0.90	0.47
Benzene	2.43	-	0.88	0.89	0.45
Toluene	2.78	-	0.85	0.83	0.37
Ethylbenzene	3.19	-	0.84	0.80	0.31

*: Ratio of retention ratio (*k*); Eluent: Mixture of 50 mM sodium phosphate solution (pH 3.0) and methanol (1+1). Column temperature: 37 °C; Column size: 50 x 2.1 mm i.d.; Flow rate: 0.2 mL min^{-1}.

Hydrophilic Interactions (HI) include interactions excluding the hydrophobic interactions. Hydrophobic interactions can be understood by obtaining van der Waals energy values using Molecular Mechanics (MM) calculations. Hydrophilic interactions can also be obtained by determining hydrogen bonding and electrostatic energy values using MM calculations. HI occurs in both aqueous and non-aqueous conditions. In liquid chromatography, the elution of analytes requires strong, generally, polar solvents. Hydrophobic solvents such as n-hexane in normal-phase liquid chromatography and less hydrophilic solvents such as acetonitrile are used for the dilution of the strong solvent to control the molecular interactions between the analytes and the packing materials, including the capillary column wall. These dilution solvents increase the retention of analytes. Therefore, polar compounds adsorb on the surface of polar adsorbents in eluents containing high concentration of organic modifiers. The phenomenon can be easily classified from reversed-phase liquid chromatography. However, according to a published report [5], the quantitative explanation of the retention mechanism in HI liquid chromatography is inconclusive. When the experimental conditions are analyzed, the chromatographic conditions are found to be unstable and unreproducible; this arises because many packing materials are neither stable nor guaranteed for long-term use by the manufacturers. When we consider a theoretical analysis of the retention mechanisms, we must use inert and stable packing materials guaranteed by the manufacturers for long-term use and exchange of the experimental data. Here, the experimental measurements of inert and stable bonded-phase silica gels made from pure porous silica gel are quantitatively analyzed *in silico* to understand the retention mechanisms of HILIC mode chromatography.

Experimental

In reversed-phase liquid chromatography, the elution order is basically related to the octanol-water partition coefficient (log P) of the analytes [9], as well as the influence of the acid dissociation constant (p*Ka*) [10]. Therefore, the retention behaviors of benzene, benzoic acid and phenol were studied in reversed-phase mode liquid chromatography using Pentyl (C5), Octyl (C8) Hexenyl (Hx), Hexylamino- and Hexylguanidino-bonded silica gels that were end-capped, and are inert according to the chromatographic behavior of pyridine [1]. The molecular properties are summarized in (Table 1). The retention times of benzoic acid, phenol, 4-chlorophenol, 2,4-dichlorophenol, 2,4-6-trichlorophenol, benzene, toluene, and ethyl-benzene were measured in a mixture of 50mM sodium phosphate solution (pH 3.0) and methanol (1+1) at 37°C. The column size was 50 x 2.1 mm i.d. and the flow rate was 0.2 mL min^{-1}. The void volume standard compound was fructose [9]. We calculated the ratio (*k*1/*k*2) of retention ratio (*k*) measured using the polar columns (*k*1) against that measured using C8 column (*k*2); the results are summarized in (Table 1).

Simple model phases (Figure 1) and the analytes are constructed using a molecular modeling program, and the Molecular Interaction (MI) energy values were calculated using the following equations. The HB, ES, and VW energy values were calculated using the CAChe MM program (Fujitsu, Japan). The computer was a PC with Intel Core i7 from LG. These MI energy values (kcal mol^{-1}) are the sum of solute and model phase energy values minus a complex energy value, were calculated per the following equations [1]. MIHB, MIES, and MIVW are MI energy of HB, ES, and VW energy values, respectively.

MIHB = HB (molecule-A) + HB (molecule-B) – HB (molecule-A and molecule-B complex), MIES = ES (molecule-A) + ES (molecule-B) – ES (molecule-A and molecule-B complex), and MIVW = VW (molecule-A) + VW (molecule-B) – VW (molecule-A and molecule-B complex). The relative MIHB, MIES, and MIVW values indicate the contribution level.

The stereo structures of model phases (molecules) are shown in Figure 1. The docking of an analyte with a model phase was achieved based on side-by-side docking that demonstrated the lowest energy values. The polar group of analytes contacted with the polar group of the model phase. The calculated MI energy values are summarized in (Table 2).

Table 2: Molecular interaction energy values of analytes with four bonded-phase silica gels.

Bonded-phase Chemicals	Octhyl-(C_8)			Hexenyl-(Hx)			Amino-(C_6NH_2)			Guanidyl-(C_6Gua)		
	MIHB	MIES	MIVW	MIHB	MIES	MIVW	MIHB	MIES	MIVW	MIHB	MIES	MIVW
Benzoic acid	0.002	0.045	7.218	1.671	0.152	7.058	23.731	12.870	2.457	33.253	0.038	0.436
Phenol	0.005	-0.006	6.262	1.708	0.018	5.688	21.398	2.112	2.181	19.597	0.511	2.632
4-Chlorophenol	0.008	0.068	7.243	1.761	0.136	6.620	20.705	-0.231	2.454	20.946	0.145	2.663
2,4-dichlorophenol	0.010	0.120	7.877	1.631	0.251	7.338	21.453	-0.478	3.545	22.565	0.917	3.023
2,4,6-Trichlorophenol	0.015	0.210	8.719	1.621	0.242	7.947	22.274	-1.238	3.981	24.505	0.575	3.921
Benzene	0	0	5.918	0	0	5.677	9.102	0	2.018	12.979	0.044	2.088
Toluene	0	-0.010	6.815	0	0.006	6.355	9.098	0.464	2.321	13.175	0.301	2.501
Ethylbenzene	0	-0.008	7.468	0	-0.009	8.369	9.084	0.482	2.380	13.208	0.246	3.161

MIHB, MIES, MIVW: Molecular interaction energy value of hydrogen bonding, electrostatic, and Van der Waals, respectively; unit: kcal mol^{-1}

Citation: Hanai T. Quantitative *In Silico* Analysis of HILIC Retention Mechanisms. Ann Chromatogr Sep Tech. 2017; 3(1): 1031.

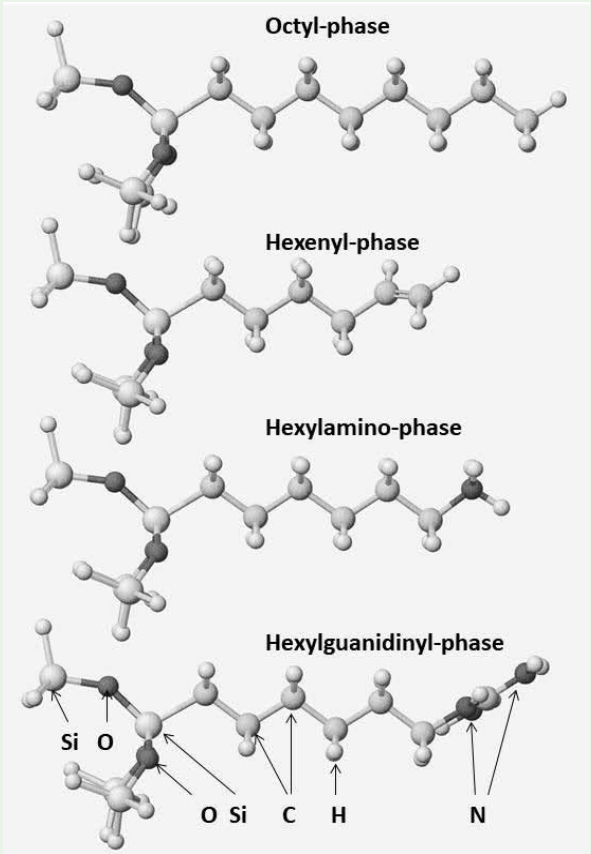

Figure 1: Stereo structure of model phases.
Atomic size: 20% of original size.

Results and Discussion

In general, the retention times of these compounds with HILIC mode decreased with increasing organic modifier concentration and with increasing polarity of the bonded phase. The ratios of the *k* values (Hx/C8) of benzene, toluene, and ethyl-benzene are less than 0.9. The results indicate that the Hx phase is more polar than the C8 phase. However, those of benzoic acid and phenol are greater than 1.0. The result indicates the existence of a specific molecular interaction between these analytes and the Hx-phase without the silanol effect. That is, the hydrophobic substitute of these analytes form contacts with the ligands of the Hx-phase by van der Waals force in less concentrated organic modifiers in reversed-phase liquid chromatography; however, the polar sites of these analytes are in contact with the polar groups of the Hx-phase by contact charge-transfer complex formation in highly concentrated organic modifiers. The latter case is called hydrophilic interaction liquid chromatography. Such polar group interactions are also observed in non-aqueous phase liquid chromatography (normal-phase liquid chromatography, previously known as adsorption liquid chromatography).

On the other hand, increasing the polarity of the bonded-phases from hexenyl- to amino-and, then, guanidyl-groups increased the retention of benzoic acid. However, increasing the polarity of the bonded-phases decreased the retention of non-polar benzene, toluene, and ethyl-benzene. The phenolic compounds demonstrated weak interaction with amino-phases, but not with the quaternary anion exchanger guanidine-phase. The above simple experiment demonstrated the retention mechanism of polar phases. Further studies were carried out using a computational chemical method (*in silico*).

These model phases are not densely bonded phases and are single molecules; therefore, the analyte can form contacts with the siloxane base. The calculated MI energy values cannot be correlated with the measured *k* values; however, the calculated MI energy values indicate the contribution of MIHB, MIES, and MIVW. The van der Waals interaction is the main interaction on the C8 phase. The MIVW values of these analytes were more than 6 kcal mol^{-1}, and the MIHB values were very low (less than 0.02 kcal mol^{-1}). The MIES values were less than 0.2 kcal mol^{-1}.

On the Hx-phase, the MIVW values of these compound were from 6 (phenol) to 8 $kcalmol^{-1}$ (2,4,6-trichlorophenol). The van der Waals force is the predominant force for the retention of these compounds. In addition, the MIHB values were about 1.7 kcal mol^{-1} for benzoic acid and phenolic compounds. Therefore, the Hx/C8 ratio of benzoic acid was 1.3, and that of phenol was 1.1 kcal mol^{-1}. The MIHB values supported the retention of compounds with polar compounds retained on the Hx-bonded phase with hydrophilic interaction (hydrogen bonding) liquid chromatography.

The amino group of the amino (C_6NH_2)-phase is ionized at pH 3.0 because the dissociation constant (pKa) of propyl and octadecyl amine is 10.60 [10]. The MIHB values of these acidic compounds were more than 21 kcal mol^{-1}, and those of neutral compounds were less than 9 kcal mol^{-1}. In particular, the combined MIHB and MIES value of benzoic acid was the highest (37 kcal mol^{-1}). This result indicates the relatively high retention time of benzoic acid on this C_6NH_2-phase. The MIES of benzoic acid was very high (13 kcal mol^{-1}), whereas those of the other compounds were less than 3 kcal mol^{-1}. The MIVW values were less than 4 kcal mol^{-1}. These results indicate that the main interaction force on the amino phase is hydrogen bonding. On the guanidinyl (C6Gua)-phase, the main interaction force was hydrogen bonding, and the MIHB values of benzoic acid, phenolic compounds, and neutral compounds were 33, 21, and 13 kcal mol^{-1}, respectively. The maximum MIVW value was 3.9 kcal $mol^{-1}$of 2,4,6-trichlorophenol. The MIVW of benzoic acid was only 0.4 kcal mol^{-1}. These values also support the chromatographic results, where only benzoic acid was highly retained on the C6Gua-phase.

Conclusion

The relative retention times (*k*) of acidic and neutral compounds were measured using reversed-phase mode liquid chromatography, an acidic eluent, and four different bonded-phase silica gels. The *k* values measured using the octyl-bonded phase were used as the standard and compared with the *k* values measured in polar phases. Benzoic acid and phenol were retained on the polar phases. The selectivity of the polar phases is supported by the molecular interaction energy values calculated using a molecular mechanics program. These compounds were retained at the alkyl-ligands of these bonded-phases by van der Waals force and the polar groups by hydrogen bonding. The difference in the molecular interaction strengths were quantitatively analyzed using the calculated energy values.

Citation: Hanai T. Quantitative *In Silico* Analysis of HILIC Retention Mechanisms. Ann Chromatogr Sep Tech. 2017; 3(1): 1031.

References

1. Hanai T. Quantitative in silico chromatography: Computational modeling of molecular interactions, Royal Society of Chemistry, Cambridge, UK. 2014; 1-338.
2. Mc Calley DV. Study of the selectivity, retention mechanisms and performance of alternative silica-based stationary phases for separation of ionized solutes in hydrophilic interaction chromatography, J. Chromatogr. A. 2010; 1217: 3408-3417.
3. Karatapanis AE, Fiamegos YC, Stalikas CD. Study of the behavior of water-soluble vitamins in HILIC on a diol column, Chromatographia. 2010; 71; 751-759.
4. Karatapanis AE, Fiamegos YC, Stalikas CD. A revisit to the retention mechanism of hydrophilic interaction liquid chromatography using model organic compounds. J. Chromatogr. A. 2011; 1218: 2871-2879.
5. Jandera P. Stationary and mobile phases in hydrophilic interaction chromatography: A review, Anal. Chim. Acta. 2011; 692; 1-25.
6. Jandera P, Janas P. Recent advances in stationary phases and understanding of retention in hydrophilic interaction chromatography. Anal. Chim. Acta. 2017; 967; 12-32.
7. Noga S, Bocian S, Buszewski B. Hydrophilic interaction liquid chromatography columns classification by effect of solvation and chemometric methods. J. Chromatogr. A. 2013; 1278: 89-97.
8. Schuster G, Lindner W. Additional investigations into the retention mechanism of hydrophilic interaction liquid chromatography by linear solvation energy relationships, J. Chromatogr. A. 2013; 1301: 98-110.
9. Hanai T. HPLC a Practical Guide, Royal Society of Chemistry, Cambridge. 1999; 134.
10. David R. Lide. CRC Handbook of Chemistry and Physics, CRC Press. Boca Raton. 1995.

Citation: Hanai T. Quantitative *In Silico* Analysis of HILIC Retention Mechanisms. Ann Chromatogr Sep Tech. 2017; 3(1): 1031.