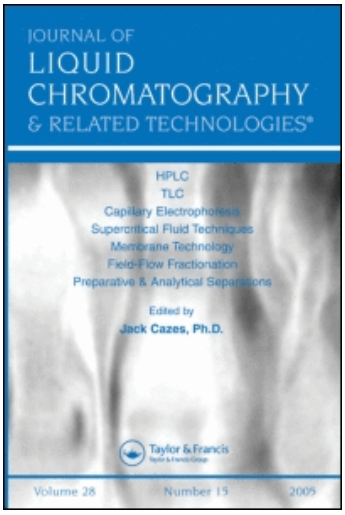

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Chromatography *In Silico*; Retention of Basic Compounds on a Carboxyl Ion Exchanger

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Chromatography *In Silico*; Retention of Basic Compounds on a Carboxyl Ion Exchanger

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Abstract: Chromatographic behavior of basic compounds, including basic drugs, in ion-exchange liquid chromatography was analyzed *in silico* to develop quantitative structure retention relationship models. The determination of molecular interaction energy values between an analyte and a model-phase, determined using a direct molecular mechanics calculation, demonstrated that retention order could be predicted, even in ion-exchange liquid chromatography with a pH controlled eluent.

Keywords: Chromatography *in silico*, Basic compounds, Ion-exchange liquid chromatography, Computational chemistry, p*K*a

INTRODUCTION

Quantitative structure retention relationship (QSRR) models require further optimization. The octanol-water partition coefficient (log *P*) was first demonstrated to be a quantitative molecular property of analytes in reversed-phase liquid chromatography,[1] and later, log *P* values were used for the quantitative analysis of chromatographic optimization.[2] A similar approach is commercialized by the Advanced Chemistry Development Laboratory.[3] Log *P* is a property of the molecular form, but not the ionized form, of compounds. Therefore, the use of log *P* induces errors in the predicted retention times of

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ionized compounds. A new approach was proposed based on direct calculation of molecular interaction energy values between a model phase and an analyte *in silico.* This approach has been examined for reversed-phase liquid chromatography of various compounds, i.e., phenolic compounds,[4,5] benzoic acid derivatives,[6] acidic drugs,[7] and basic drugs.[8] The correlation coefficient between measured and predicted retention factors was equivalent or better than that obtained using the log *P* system. Especially, analytes whose log *P* values of the analytes' fragments are not established cannot be handled by the log *P* system with good precision. The new method can be applied to estimate the relative retention factor of newly designed compounds, because this system does not require experimental data to predict the elution order. The precision of this new approach was less than that optimized by DryLab (computer-assisted method),[9] but the theoretical approach should open a new dimension to quantitatively study molecular interactions and to design new phases. In this report, a new bonded-phase was synthesized for the development of fast HPLC screening of basic drugs in a drug discovery process. The chromatographic behavior of the new bonded-phase for basic drugs was analyzed quantitatively *in silico.*

EXPERIMENTAL

Preparation of Hexylcarboxyl Bonded Silica Gel

A hexylcarboxyl-bonded silica gel was synthesized from a hexenyl-bonded silica gel instead of binding 5-bromo-1-pentene with trichlorosilane due to the availability of the silyl-reagent.[10] Hexenyltrichlorosilane (1.1 mL) from Nacalai Tesque (Kyoto, Japan) and 2 g of 5 μm silica gel (MS Gel EP-DF 100, 100 Å pore size) from Dokai-Kagaku (Kitakyushu, Japan) were mixed in toluene; then the mixture was refluxed. The hexenyl-bonded silica gel was bromated using 30% hydrogen bromide (HBr) in acetic acid from Sigma-Aldrich Corporation (St. Louis, MO). The bromohexyl-bonded silica gel was oxidized in dimethylsulfoxide (DMSO) (Komblum oxidation). The aldehyde form was further oxidized to the carboxyl-form by potassium permanganate oxidation in 50% aqueous acetone. The total organic content of the bonded-silica gel was 15.5 wt%; the ion-exchange capacity was 75 μEq/g by titration.

Measurement of Retention Factors

Drugs and chemicals were obtained from Sigma-Aldrich Corporation and Wako Pure Chemicals (Osaka, Japan). Their properties are summarized in Table 1. Acetic acid, sodium acetate, and sodium hydroxide were purchased

Table 1. Properties of basic compounds

No.	Chemicals	p*K*a1	p*K*a2	p*K*a3	ΔFSm	ΔFSi	hbm	hbi
1	Ajmaline	8.2	5.753	5.556	30.4227	31.7423	−2.317	−2.110
2	Aniline	4.69	3.818	3.408	14.2624	18.8636	−1.334	−1.334
3	Atropine	9.8	6.555	6.536	20.7190	23.0025	−3.451	−3.387
4	Dextromethorphan	8.3	6.184	5.618	27.6929	36.2590	0	0
5	Homatropine	9.9	6.413	6.597	26.0110	23.8780	−0.606	−3.306
6	Imipramine	9.5	6.231	6.352	27.3559	39.3437	0	0
7	Isoproterenol	8.6	6.005	5.801	19.1121	26.3668	−11.250	−8.581
8	Lidocaine	7.9	5.735	5.373	22.2696	28.1267	−2.406	−2.226
9	Prazosin	6.5	4.147	4.516	26.9866	24.2851	−1.257	−1.099
10	Pyridine	5.23	3.463	3.739	14.6571	17.0381	0	0
11	Quinine	8.5	5.745	5.740	29.9987	34.4376	−4.612	−4.621
12	Scopolamine	7.75	4.801	5.281	22.2733	23.4282	−4.157	−4.146
13	Terbutaline	8.8	5.912	5.924	24.2510	25.1261	−6.111	−5.812
14	Triamterene	6.2	3.851	4.332	21.7167	27.5352	−3.085	−2.844
15	Benzylamine	9.33	6.079	6.248	15.2344	21.4438	−0.973	−0.983
16	Phenylethylamine	9.82	6.650	6.548	17.5244	23.6096	−0.089	−0.089
17	N,N′-Dimethylaniline	5.15	3.935	3.690	20.9785	25.5160	0	0

p*K*a1: reference values; p*K*a2: measured by ion-exchange liquid chromatography; p*K*a3: predicted from the relation between p*K*a1 and p*K*a2; ΔFSm: ΔFS of molecular form corrected with hbm; ΔFSi: ΔFS of ionized form corrected with hbi; hbm: hydrogen bonding energy of molecular form analyte; hbi: hydrogen bonding energy of ionized analyte; energy unit: kcal/mol.

from Wako Pure Chemicals. HPLC grade methanol was obtained from Kanto-Kagaku (Tokyo). Milli-Q grade water was used.

The automated liquid chromatograph consisted of a G1310A IsoPump, a G1313A ALS auto-sampler, and a 359500E Interface, which were controlled by a host computer Model Vectra XM series 316/90 (Yokogawa Analytical Systems [Agilent Technologies], Tokyo, Japan). A Jasco model UV970 spectrophotometric detector was obtained from Jasco (Tokyo, Japan). The Sigma02 column oven was purchased from IRICA (Kyoto, Japan) and the degasser was a Degasser Populaire from Sanwa-Tsusho (Tokyo, Japan).

The carboxyl-bonded silica gel column, 50 mm × 2.1 mm i.d., was used for the ion-exchange liquid chromatography of basic compounds. The eluent was 50 mM sodium acetate solution, pH 3.20 to 9.50, containing 50% methanol. The column temperature was 37°C. The void volume marker was fructose. The flow rate was 0.2 mL/min.

Quantitative Analysis of the Retention *In Silico*

The computer used was a Dell latitude C840 equipped with a 2 GHz processor and 1024 MB memory. The molecular properties of the analytes and model phases and molecular interaction energy values were calculated by molecular mechanics (MM2) using the CAChe program (version 5; Fujitsu, Tokyo, Japan). The standard parameters used were bond stretch, bond angle, dihedral angle, improper torsion, van der Waals, hydrogen bonds, and electrostatic forces (MM2/MM3 bond dipoles). The van der Waals cut-off distance was 9 Å. The energy unit was kcal/mol (1 kJ/mol = 4.18 kcal/mol). Data analysis was performed using the Cricket-Graph program (Computer Associates, San Diego, CA) and Project Reader of the CAChe program.

To develop the QSRR in chromatography, the molecular interaction energy value (Δenergy) was calculated between model phases and analytes. The optimized energy value was less than 0.00001 kcal/mol. The Δenergy was the subtracted energy value of the complex from the sum of energy values of the model phase and analyte. The Δenergy values of molecular and ionized forms of the analytes are summarized in Table 1. The Δenergy values of the final structure (ΔFS) were correlated with the retention factors measured in liquid chromatography.

RESULTS AND DISCUSSION

Inductive Effect on pKa

The retention time was longer in the new ion-exchange liquid chromatography method. The precision was higher for the weakly retained compounds in this

liquid chromatography system. Previously, the negative log k values were not included in the analysis due to the very short retention time.[10] The retention time was reasonably long on this new carboxyl-bonded phase, however, and all measured retention factors could be included in the analysis.

Furthermore, the dissociation constant (p*K*a) of these analytes was calculated from the retention factors measured using eluents of pH 3.2 to 9.5, and the retention factors are given in Fig. 1. Allopurinol, amoxicillin, carbamazepine, diazepam, procaine, theobromine, and theophylline did not behave as basic components in the chromatograph; therefore, these compounds are not included in Table 1 and were eliminated from further analysis. The p*K*a values obtained from the experiment were very different from those in the literature listed in Table 1. The difference was partly influenced by the methanol concentration in the eluent, but the inductive effect between the carboxyl group of the ion-exchanger and the analyte might also contribute to the difference. The relationship between measured and reference values is given as the following equation:

$$\text{p}K\text{a (ion-exchange LC)} = 0.612\ (\text{p}K\text{a reference}) + 0.538,$$
$$\text{r} = 0.960, \quad n = 17, \tag{1}$$

The p*K*a of aromatic acids measured using a propylamino-bonded silica gel (NH2) was smaller than that measured using an octadecyl-bonded silica gel (ODS), and that measured using diethylaminoethyl-bonded silica gel

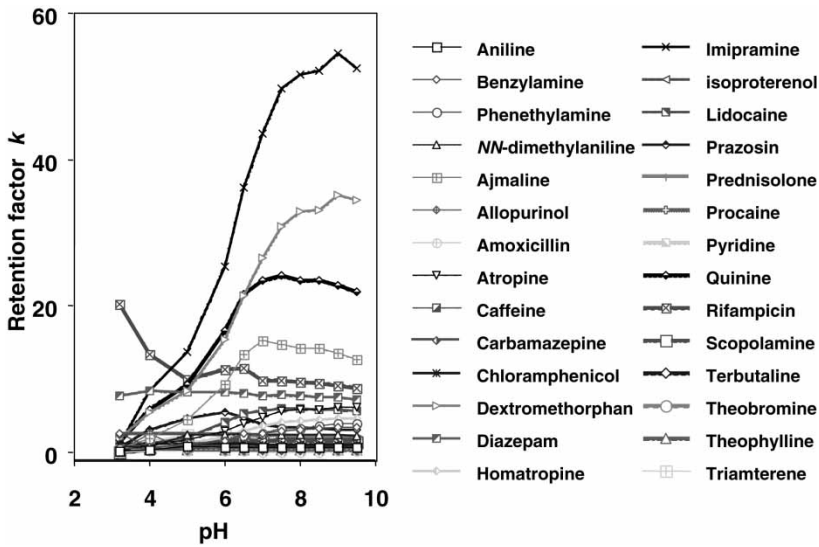

Figure 1. pH effect for the retention of basic compounds in ion-exchange liquid chromatography using carboxyl-phase. Chromatographic condition: see in text.

(DEAE) was also smaller. The p*K*a measured using a cation exchanger (SP) was shifted to a higher pH region.[11] The relationships are given in the following equations:

$$\text{p}K\text{a (NH2)} = 0.744\ (\text{p}K\text{a (ODS)}) + 0.446, \quad r = 0.988, \quad n = 29 \qquad (2)$$

$$\text{p}K\text{a (DEAE)} = 0.847\ (\text{p}K\text{a (ODS)}) + 0.268, \quad r = 0.990, \quad n = 29 \qquad (3)$$

$$\text{p}K\text{a (SP)} = 1.023\ (\text{p}K\text{a (ODS)}) - 0.097, \quad r = 0.998, \quad n = 29, \qquad (4)$$

A total of 36 aromatic acids was used; however, 7 aromatic acids were eliminated to determine the properties of ion-exchangers. The eliminated aromatic acids demonstrated the possibility of intra-molecular hydrogen bonding.

These data were measured under the same conditions containing 20 v% acetonitrile. Therefore, the concentration effect of an organic modifier could be neglected for comparison. The effect of an organic modifier in the reversed-phase liquid chromatography was approximately 0.022 pH unit/% of methanol or acetonitrile, experimentally.[12,13] The concentration effect of the organic modifier depended on the buffer components, even when the pH was measured using standard buffers for a pH meter with the same concentration as the organic modifier. Furthermore, the p*K*a shift in the aqueous organic solvent depended on the analyte itself.[14] This organic modifier effect was observed for carboxylic acid in reversed-phase liquid chromatography[15] and for polar aromatic compounds on cation-exchanger and porous polymer gels.[16] The difficulty of standardizing the organic modifier effect is due to the difficulty in performing quantitative analysis of solvation. In this experiment, however, pH was measured before mixing with the organic modifier; therefore, the relatively higher pH values are a likely property of the ion-exchanger used.[11]

The slope of equation (1) indicates the selectivity of the carboxyl-phase. The inductive effect of the ion-exchanger affects the shift of the p*K*a values measured, and the degree of the shift might depend on the ion-exchange capacity and the ionic strength, and also the ionic strength of buffer components of the eluent. The ion-exclusion effect at higher and lower pH regions was higher in sodium-phosphate solution than that in sodium-acetate solution.

Quantitative Analysis of Log k *In Silico*

Using a computational chemical calculation to analyze liquid chromatographic data, the direct interaction between a model-phase and an analyte was calculated as energy values using the molecular mechanics (MM2) calculation quantitatively. The quantitative analysis of retention in liquid chromatography is easy, because a homogeneous model phase can be used instead of a complicated protein model for studying docking mechanisms.

For QSRR of reversed-phase liquid chromatography, the contact surface area is important, and the selection of a model phase is difficult, but a simple model phase is satisfactory for the measurement of albumin-acidic drug binding affinity in ion-exchange liquid chromatography.[17] A simple model carboxyl-bonded phase was constructed to investigate basic compound-carboxyl phase interactions. The model phase consisted of 556 carbons, 48 oxygens, 957 bonds, and 5448 connectors. The molecular weight was 7440. The 24 carboxyl groups were in 390 $Å^2$. The optimized energy value was less than 0.00001 kcal/mol. The 1:1 adsorption form of quinine on the carboxyl phase is shown in Fig. 2 as an example of the adsorption of a basic compound on the carboxyl phase.

The pH effect on molecular interaction can be examined experimentally using liquid chromatography. The retention factor in a given pH eluent can be predicted from the following equation:[18]

$$k = (k\mathrm{m} + k\mathrm{i}([\mathrm{H}^+]/[K])/(1 + ([\mathrm{H}^+]/[K]), \quad (5)$$

where *k*m and *k*i are retention factors of the molecular and ionized forms of the analytes, H^+ is the concentration of hydrogen ions in the eluent, and *K* is the dissociation constant of the analyte. The *k*m and *k*i were replaced by molecular interaction energy values calculated using molecular mechanics. The *k*m value was replaced with the Δenergy value of the molecular form of the analyte (ΔFSm) and the *k*i value was determined by the Δenergy value of the ionized form of the analyte (ΔFSi). The following equation is used for further discussion:

$$\Delta\mathrm{FS} = (\Delta\mathrm{FSm} + \Delta\mathrm{FSi}([\mathrm{H}^+]/[K])/(1 + ([\mathrm{H}^+]/[K]), \quad (6)$$

It was necessary to first determine how to obtain the relative dissociation constant in ion-exchange liquid chromatography. The relative p*K*a values

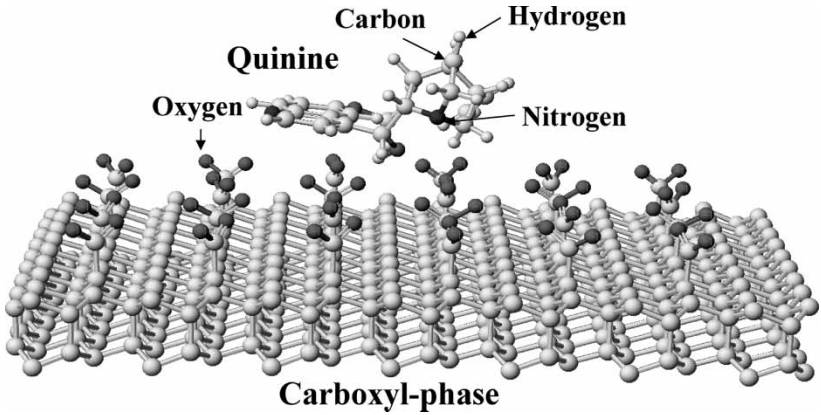

Figure 2. Docking of quinine on carboxyl-phase; small white ball: hydrogen; large white ball: carbon; large gray ball: oxygen; large black ball: nitrogen.

measured in this ion-exchange liquid chromatography were shifted to lower values compared to the reference values. The original p*K*a values measured by titration were affected by the organic modifier concentration and the inductive effect of ion-exchange groups of the bonded phase. ΔFS was calculated using equation (6).

The molecular interaction energy values were correlated with log k values. There was a poor correlation, however, between the ΔFS and log k values (data not shown). The electrostatic energy values were the major contributors for acidic drugs, and the hydrophobic interaction was important for basic drugs.[19] Therefore, a new model phase was constructed to increase the contact surface area for hydrophobic interactions. The model phase was based on a model phase constructed to study the ionization effect of the docking of an acidic drug with protein.[20] The structure is shown in Fig. 3, where scopolamine was docked with the model phase. One ionized carboxyl group was located at the center of the carbon-phase and six methyl groups surrounded the carboxyl group to protect against the binding of octyl groups. There was a total of 16 octyl groups. The longer neighbor alkyl chains were bound together by hydrophobic interaction, i.e., van der Waals energy, in this type of molecular modeling. The model phase consisted of 1350 atoms including 2 oxygens, 739 hydrogens, 609 carbons, 1588 bonds, and 9121 connectors. Hydrogen bonding energy did not contribute to the retention; therefore, further calculation was performed without the hydrogen bonding energy values of analytes (hb). The ΔFS was calculated by ΔFSm + hbm for the molecular form and ΔFSi + hbi for the ionized form

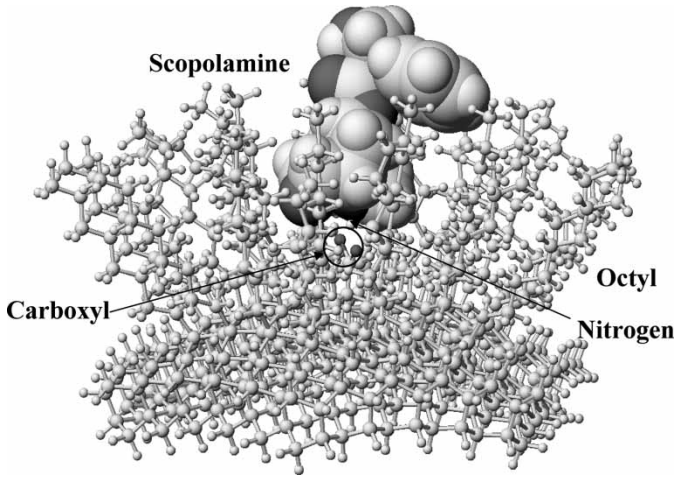

Figure 3. Docking of scopolamine with carboxyl-octyl phase; small white ball: hydrogen; large white ball: carbon; large gray ball: oxygen; large black ball: nitrogen; atom size of scopolamine is 5 times of carboxyl-octyl phase.

in equation (6). The correlation between predicted log k and the experimental data is given as the following equations:

$$\Delta\text{FS (pH 8.0)} = 7.171 \times \log k\,(\text{pH 8.0}) + 18.090,\ \text{r} = 0.766,\ \text{n} = 17, \quad (7)$$

$$\Delta\text{FS (pH 7.0)} = 8.134 \times \log k\,(\text{pH 7.0}) + 18.031,\ \text{r} = 0.845,\ \text{n} = 17, \quad (8)$$

$$\Delta\text{FS (pH 6.0)} = 10.744 \times \log k\,(\text{pH 6.0}) + 18.879,\ \text{r} = 0.913,\ \text{n} = 17, \quad (9)$$

$$\Delta\text{FS (pH 5.0)} = 12.388 \times \log k\,(\text{pH 5.0}) + 21.412,\ \text{r} = 0.823,\ \text{n} = 17, \quad (10)$$

$$\Delta\text{FS (pH 4.0)} = 11.912 \times \log k\,(\text{pH 4.0}) + 25.040,\ \text{r} = 0.800,\ \text{n} = 17, \quad (11)$$

The correlation coefficient was high around the p*K*a values of these compounds. The correlation coefficient was poor at higher pH regions, where a ion-exclusion effect reduced the retention (Fig. 1). The high ΔFSm and ΔFSi values indicated that the molecules fit well with the model phase for the computational chemical calculation.

Further study was performed using the original p*K*a values from the literature. The p*K*a of analytes was considered to be constant and not directly affected by ion-exchange groups and organic modifiers for their log k values. The correlation coefficients are given as the following equations:

$$\Delta\text{FS (pH 8.0)} = 10.777 \times \log k\,(\text{pH 8.0}) + 18.380,\ \text{r} = 0.932,\ \text{n} = 17, \quad (12)$$

$$\Delta\text{FS (pH 7.0)} = 11.760 \times \log k\,(\text{pH 7.0}) + 18.643,\ \text{r} = 0.940,\ \text{n} = 17, \quad (13)$$

$$\Delta\text{FS (pH 6.0)} = 12.429 \times \log k\,(\text{pH 6.0}) + 19.859,\ \text{r} = 0.900,\ \text{n} = 17, \quad (14)$$

$$\Delta\text{FS (pH 5.0)} = 12.333 \times \log k\,(\text{pH 5.0}) + 22.287,\ \text{r} = 0.830,\ \text{n} = 17, \quad (15)$$

$$\Delta\text{FS (pH 4.0)} = 11.223 \times \log k\,(\text{pH 4.0}) + 25.621,\ \text{r} = 0.806,\ \text{n} = 17, \quad (16)$$

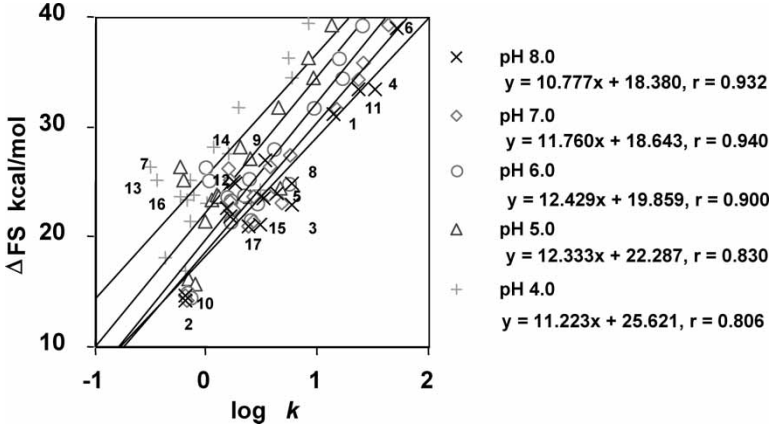

Figure 4. Relationship between interaction energy (ΔFS) and log k of retention factors at different pH eluents; numbers beside symbols: see Table 1.

These relations are shown in Fig. 4. The correlation coefficient was relatively higher than that calculated using p*K*a values obtained in this ion-exchange liquid chromatography. This result indicated that molecules were dissociated based on the individual p*K*a values. The original p*K*a values measured by titration can be used for a theoretical approach in studying molecular interaction. Measurement of the organic modifier and ion-exchange group effects is not necessary prior to the calculation.

CONCLUSION

Chromatography *in silico* using a model phase was practical for studying the retention mechanism. Furthermore, elution order can be predicted even in ion-exchange liquid chromatography. The difficulty is predicting the relative p*K*a values in ion-exchange liquid chromatography. Compounds, however, dissociated based on their p*K*a measured by titration. Computational chemical optimization based on the molecular properties of analytes is practical in liquid chromatography, and allows for the study of molecular interactions.

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