Chromatography and Computational Chemistry for Drug Discovery

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Chromatography can be used to measure molecular properties and interactions (e.g. log $P$, $pK_a$, protein-drug binding affinity, chiral recognition). Computational chemistry can be used to estimate molecular properties and interactions (log $P$, $pK_a$, NMR, IR, absorption spectra, chiral recognition mechanisms, molecular recognition mechanisms). Chromatography can pick up a target compound from crude mixtures, and its structure can be determined by instrumental analyses such as NMR, MS, absorption spectra, ICP, X-ray crystallography etc. Computational chemistry can suggest a target compound from expected compounds based on estimated molecular properties and interactions. A combination of chromatography and computational chemistry will accelerate drug discovery.

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QS RR

Log $P$ and $pK_a$ estimation methods were developed by computational chemical methods based on retention factors determined by reversed-phase liquid chromatography. Then, the quantitative structure retention relationship (QSRR) in reversed-phase liquid chromatography in eluent at a given pH can be developed based on the new log $P$ ($N\log P$) and $pK_a$ values. A CAChe log $P$ method was modified to determine QSRR in reversed-phase liquid chromatography. Previously, the modified CAChe log $P$ ($N\log P$) was used to predict retention factors of molecular form compounds such as phenolic compounds and nitrogen-containing compounds by the same equation instead of requiring individual equations for different groups of compounds$^7$. This method was successfully applied for QSRR of molecular form aromatic acids.

$$k = y \times \log P + m$$

where $k$ is retention factor of molecular form compounds, $y$ and $m$ are constants in a given system.

Furthermore, the prediction of dissociation constants ($pK_a$) was studied using atomic partial charges calculated by CAChe MM2/AM1 programs. The $pK_a$ values of various aromatic acids and their derivatives were predicted by simple equations utilizing the partial charge of hydrogen of the carboxyl groups instead of using individual Hammet’s equations and $\sigma$ constants$^7$ as applied previously.$^7$

$$pK_a = A + B\Sigma\sigma_i$$

where $A$ and $B$ are constants for individual groups of compounds, and $\sigma$ is Hammet’s $\sigma$ constant. In new method,

$$A = -210.485 \times (\text{partial charge}) + 55.797$$

$$B = -12.412 \times \Delta(\text{partial charge of sustituent}).$$

A combination of hydrophobic interactions related to $N\log P$ values and ionization related to $pK_a$ values made it possible to predict retention factors of aromatic acids in eluent at a given pH in reversed-phase liquid chromatography.

$$k = [k_m + k_i(\frac{K_a}{[H^+]})]\div(1 + \frac{K_a}{[H^+]})$$

where $k_m$ is the retention factor of the non-ionized acid and can be obtained from log $P$ as above and $k_i$ is the retention factor of the 100% ionized acid. The $k_i$ value cannot be predicted mathematically at present, but the value is close to zero in many cases. The example of relation between predicted and measured retention factors of aromatic acids in reversed-phase liquid chromatography using an octadecyl-bonded silica gel and different pH eluents is shown in a following Figure 1.

$$y = 0.768x + 0.024 \quad r^2 = 0.98$$

$$y = 0.656x + 0.029 \quad r^2 = 0.98$$

$$y = 0.752x - 0.038 \quad r^2 = 0.988$$

$$y = 0.815x - 0.127 \quad r^2 = 0.984$$

$$y = 0.885x - 0.207 \quad r^2 = 0.98$$

Fig. 1 Relations between predicted and measured retention factors.

This new prediction method of retention factor is an advanced simple method, compared with the old method used the empirical calculation methods of Rekker’s log $P$ and Hammett’s $pK_a$. This QSRR can be used for the analysis of metabolites and quality control of drugs.

Drug-albumin binding affinity

Albumin-acidic drug binding affinity ($\log nK$) measured by a modified Hummel-Dreyer method (liquid chromatography) was predicted without albumin from retention factors measured.
by reversed-phase liquid chromatography using butyl- and phenylhexyl-bonded silica gel and ion-exchange liquid chromatography using guanidyl-bonded packing materials.  

\[
\log nK = 1.231 \{1.00 \log k (HB) + 0.70 \log k (IX) - 0.20 \log k (\pi)\} + 5.175, r^2 = 0.948,
\]

where \( nK \) is binding affinity, \( \log k (HB) \) are \( \log k \) values measured by reversed-phase liquid chromatography at pH 7.4, \( \log k (IX) \) are the \( \log k \) values measured by ion-exchange liquid chromatography at pH 7.4, and \( \log k (\pi) \) are \( \log k \) values measured by reversed-phase liquid chromatography using phenyl-phase at pH 7.4.  

This equation for acidic drugs was improved by new systems. Furthermore, albumin-basic drug binding affinity was predicted without albumin from retention factors measured by reversed-phase liquid chromatography using pentyl-bonded silica gel and ion-exchange liquid chromatography using carboxyl-bonded silica gel.  

\[
\log nK = 2.614 (\log k (R) + 0.453 \log k (I)) + 3.120, r^2 = 0.949,
\]

for acidic drugs, and  

\[
\log nK = 0.603 (\log k (R) + \log k (I)) + 3.317, r^2 = 0.987
\]

for basic drugs, where \( \log k (R) \) was \( \log k \) measured on reversed-phase using pentyl-bonded silica gel and \( \log k (I) \) was that determined on ion-exchange liquid chromatography using either guanidyl-bonded phase or carboxyl-bonded phase.  

The reference \( nK \) values varied significantly, probably due to different qualities of human serum albumin and different analytical systems used. The \( \log nK \) values measured by the modified Hummel-Dreyer method using purified human serum albumin were correlated well with \( \log k \) values measured by reversed-phase liquid chromatography using pentyl bonded silica gel column and ion-exchange liquid chromatography using guanidino-bonded silica gel and carboxyl-bonded silica gel columns. These will be a simple and faster experimental screening method to measure drug-protein binding affinity without albumin with great reproducibility.  

**Chiral recognition**  

Chromatographic molecular interactions including chiral recognition were analyzed by computational chemical methods using model solid phases. Popular chiral phases are called Pirkle type phases and used in normal-phase liquid chromatography. The most important molecular interaction force is hydrogen bond. The chiral recognition centers of the model chiral molecules were analyzed using the Extended Huckel CAChe program, and then the structure of hydrogen bond complexes with analytes were optimized by molecular mechanics calculations. The differences in final energy values indicated the elution order and enantiomer separation. Addition of solvent effect in computational calculation will improve the precision.  

In aqueous system, ligand-exchange liquid chromatography is applied for enantiomer separations. The enantiomer separation of Cu-complex was also analyzed by computational chemical method, and the differences in final energy values indicated the elution order and enantiomer separation. The computational chemical method will shorten a column selection time of drug enantiomer separation that becomes more important for drug discovery. Synthesis of bonded ligand-exchange phase will improve the chromatographic reproducibility.  

**Conclusion**  

A combination of chromatography and computational chemistry is a rapid and effective drug candidate screening method and shorten the development time to optimize the quality control methods. Chromatography and computational chemistry will accelerate drug discovery.  

**References**  