

Prediction of Retention in Gradient Reversed-Phase Liquid Chromatography for Phenylisothiocyanate-Derivatives of Amino Acids

ABSTRACT

The solvatic retention model of reversed-phase liquid chromatography has been used to predict retention of phenylisothiocyanate derivatives of 25 natural amino acids under conditions of gradient elution. Retention factors have been calculated from molecular parameters of structures of analytes and characteristics of a column and an eluent. Step-by-step method which includes the first guess prediction of initial conditions from structural formula and fine tuning parameters of the retention model using data of successive runs can save time in method development and optimization of separation of target compounds.

Keywords: High-performance liquid chromatography; Solvation sorption model; ChromSword computer simulation software; Amino acids; Phenylisothiocyanate derivatization.

1. INTRODUCTION

High performance liquid chromatography (HPLC) is the most frequently used method of analysis of organic compounds. Two modes of elution are used in HPLC: isocratic and gradient. Isocratic elution is suitable for the analysis of samples with a relative narrow range of retention and the gradient mode is used to separate compounds with a wide range of hydrophobicity and retention. It is common knowledge that development and optimization of HPLC gradient methods can be time consuming and requires many experiments [1,2]. Different approaches that can reduce method development and optimization time are still under consideration and studies [2,3].

Relationship between retention and molecular structure of analytes has been an interesting and important theoretical and practical problem in chromatography for a long time. Prediction of retention using chemical structures of compounds and column properties can save time and reduce number of experiments. A number of studies concerning this area have been reviewed [4-7]. Today, to simplify and accelerate the chromatographic method development process, several commercially available computer simulation software packages have been used [3,8-10] however only few contain options to predict retention from chemical structures. ChromSword uses the solvatic retention model [3,11-13] which was derived from the solvophobic retention model of RP LC [14] and ACD ChromSimulator [3,10] applies a linear relationship between the LogP predicted from structural formulae and retention in reversed-phase liquid chromatography. Some articles have been published for prediction of retention in gradient and isocratic elution from chemical structures [15-17] utilizing a generic equation: $[\text{retention}] = k_1 + k_2a_1 + \dots + k_6a_5$ where $(a_1 - a_5)$ are different molecular descriptors and $k_1 - k_6$ are coefficients to be determined from so called a "training set" of compounds. Different software (ACD, HYPERCHEM, HEMPLUS, CLOGP, etc) were used to calculate molecular descriptors [14-17]. This approach requires preliminary calibration of a chromatography system (column, mobile phase, gradient profile, etc.) using a set of 14-17 or even more [17] standard compounds to determine correlation coefficients to start prediction for other structures. Correlation coefficients cannot be used for other gradient profiles and columns and this limitation makes the approach hardly usable for practical chromatography. Correlative approaches do not consider any retention model of the reversed phase chromatography and detail discussion of those approaches is out of a topic of our article. In the recently published article an attempt was made to apply the solvophobic model for prediction of retention from structural parameters [18] however the approach in fact was described, applied substantially earlier [11-13] and realized in the commercial software [3,10,19,20].

Earlier were described the solvatic model of retention to predict initial conditions in reversed phase liquid chromatography from chemical structure and column characteristics [11-13]. The model was used for prediction of retention behavior of aromatic compounds [11-13], derivatives of triazine, phenylurea [21], linear and cyclic oligomers in polyamide-6 [22]. Those compounds are mainly neutral under condition of chromatographic and applicability of the model has not been studied for high polar and charged analytes. The aim of our work was to study applicability of the solvatic retention model for prediction of retention in gradient elution of phenylisothiocyanate derivatives of natural amino acids. Determination of amino acids in different objects and optimization of their separation is still an important

task and we consider that possibility to predict retention of these compounds from chemical structure can potentially save time for method development and optimization of their separation. However, several approaches for the prediction of the amino acids and its derivatives were studied before [23-28] the described algorithms for prediction of retention in this work is totally different. The main advantage of the proposed method is feasibility for prediction of initial gradient conditions without any experimental data with reasonable results.

Derivatization with phenylisothiocyanate (PITC) was chosen because this method has several advantages – pre-column derivatization possibilities, derivatives stability up to 48 hours (keeping samples at 5°C – 8°C), opportunity for primary and secondary amino acids detection and also it allows using the HPLC with UV detector [29-31].

2. EXPERIMENTAL DETAILS

2.1 Equipment and software

Chromatographic measurements were made on a liquid chromatographic system Alliance Waters 2695 (Waters, Milford, MA, USA) consisting of Waters 2487 UV detector, temperature controlled column and autosampler (at 6°C). Waters Pico-Tag 30.0×0.39 cm I.D., particle size 4 µm column was employed as stationary phase for all experiments. The injected sample volume was 10 µL. All chromatographic investigations were performed at 46°C with an eluent flow-rate of 1 mL min⁻¹. Sample hydrolysis and derivatization were carried out with the Pico-Tag Workstation (Waters).

To acquire chromatographic data, Empower 2 software (Waters, Milford, MA, USA) was used. For retention parameter prediction and resulting processing ChromSword computer simulation system, version 4.8.3.2010 (Merck KGaA, Darmstadt, Germany) was used.

2.2 Chemicals

Analytically pure sodium acetate trihydrate, 6 M hydrochloric acid and glacial acetic acid were obtained from Penta. Triethylamine (TEA) was obtained from Fluka and phenyl isothiocyanate (PITC) was obtained from Sigma Aldrich, both HPLC-grades. HPLC-grade acetonitrile and methanol were obtained from LabScan. Deionized water was passed through Milli-Q watersystem (Millipore).

Each mobile phase composition was prepared separately. The first eluent was prepared as follow: 19.0 g sodium acetate trihydrate dissolved in 1 L Milli-Q water, added 0.5 mL TEA, mixed and titrated with glacial acetic acid to pH 6.40. The solution was filtered through 0.45 µm filter. Finally 940 mL of the resulting solution was added to 60 mL CH₃CN. The second eluent was prepared from CH₃CN and water in a ratio 3:2. The eluents were degassed with sonification.

Laboratory prepared amino acids in 0.1 M HCl solution, composed from 25 commercial available amino acids (Sigma), were used. For more convenient reading the abbreviation of used amino acids are given in Table 1.

Table 1. The composition of 25 amino acid mixture, numbered according experimentally found HPLC elution sequence of PTC derivatives.

No.	Amino acid	Abbreviation*
1	Aspartic acid	Asp
2	Glutamic acid	Glu
3	α-Aminoadipic acid	Aad
4	Asparagine	Asn
5	Serine	Ser
6	Glutamine	Gln
7	Glycine	Gly

8	Histidine	His
9	Citrulline	Cit
10	Arginine	Arg
11	Taurine	Tau
12	γ-Amino butyric acid	Gaba
13	Threonine	Thr
14	Alanine	Ala
15	γ-Aminoisobutyric acid	Baib
16	Proline	Pro
17	α-Aminobutyric acid	Aab
18	Tyrosine	Tyr
19	Valine	Val
20	Methionine	Met
21	Isoleucine	Ile
22	Leucine	Leu
23	Phenylalanine	Phe
24	Tryptophane	Trp
25	Ornithine	Orn

* Abbreviations according to IUPAC recommendations

For experimental work amino acid sample was prepared by using phenylisothiocyanate derivatization and the elution pattern was recorded at 254 nm [33-35].

3. RESULTS AND DISCUSSION

To calculate approximately the retention, the solvatic retention model of reversed-phase chromatographic system was employed. The model was described in detail [11-13] and an equation for calculating retention in reversed-phase chromatography and calibration of columns was derived:

$$\ln k_x = aV_x^{2/3} + b\Delta G_{e.s.x.H_2O} + c \quad (1)$$

Where, $\Delta G_{e.s.x.H_2O}$ is energy of electrostatic interaction of analyte with water, V is partial molar volume of substance in water which determines a value of energy to create a cavity in a mobile and in the stationary phases, $a = 16.48(\gamma_m - \gamma_s)$ coefficient, where γ_m and γ_s are the surface tension of a mobile and stationary phase correspondently, $b = 0.8234 \times [f(\epsilon_m) - f(\epsilon_s)]$ coefficient, where ϵ_m and ϵ_s are dielectric permittivity of a mobile and stationary phase correspondently and c is a parameter which includes phases ratio and some other characteristics of the stationary and mobile phase.

The approach of the computer-aided method development utilizes the Eq.1 to calculate the retention in reversed-phase or HPLC from chemical structure and column properties for prediction of initial gradient conditions (a first guess linear gradient), and then find tuning the model after input of data of successive experimental runs. Such step-by-step method from the zero approximation (no run data available) to the first and second approximation (data of the first and then second run are available) can potentially save time for method development and optimization. The method requires that both parameters of the solutes – the volume and energy of interaction with water – and the characteristics of

the reversed phase column under experimental conditions to be known. The goal of the first experiment in computer-assisted method development is to provide practically reasonable retention time values for target compounds. Initial conditions – a first guess gradient method with initial, final concentration of an organic solvent in a mobile phase and a gradient time can be predicted from chemical structure and characteristic of columns with the commercially available ChromSword software [3,9]. This software contains also a data base of column characteristic of many commercial available reversed-phase columns – a, b, c coefficients in the Eq. 1 at any concentration of acetonitrile and methanol in a mobile phase.

Comparison of experimental and predicted retention time for amino acid derivatives calculated from chemical structure and column properties to provide retention in the range of 3 – 30 min are illustrated in the Table 2. We consider the prediction is quite reasonable and the approach can be used to predict a practical acceptable retention times for mixtures of phenylisothionate derivatives of amino acids.

Table 2. A comparison of predicted and experimental retention times for 30 and 60 min linear gradients

No	Amino acids	Predicted retention time, min (30 min)	Experimental retention time, min (30 min)	Predicted retention time, min (60 min)	Experimental retention time, min (60 min)
1	ASP	2.75	2.91	2.83	2.91
2	GLU	4.71	3.19	5.85	3.19
3	AAD	5.89	3.80	8.05	3.81
4	ASN	3.74	5.04	4.23	5.31
5	SER	3.59	5.26	3.94	5.57
6	GLN	4.87	5.26	6.18	5.57
7	GLY	6.37	5.57	8.33	5.99
8	HIS	8.29	5.76	12.49	6.38
9	CIT	11.09	5.95	17.47	6.70
10	ARG	3.48	6.38	3.89	7.22
11	TAU	6.34	6.38	8.65	7.22
12	GABA	9.78	6.54	14.88	7.48
13	THR	4.81	6.71	5.96	7.81
14	ALA	8.14	7.00	11.68	8.16
15	BAIB	9.73	7.00	14.78	8.28
16	PRO	10.98	7.31	17.12	8.87
17	AAB	9.73	8.58	14.78	11.05
18	TYR	9.96	9.40	15.77	12.81
19	VAL	11.05	10.37	17.39	14.48

20	MET	11.00	10.86	17.38	15.40
21	ILE	12.26	12.34	19.75	18.36
22	LEU	12.26	12.55	19.75	18.77
23	PHE	12.71	13.51	20.74	20.72
24	TRP	12.11	13.79	19.83	21.31
25	ORN	10.06	13.95	16.22	21.62

The goal of the next experiments is to predict retention time much more precisely to start optimization of separation. Using retention data obtained after the first linear gradient run, structure formula and column characteristics, it is possible to correct either value of the energy of interaction ($\Delta G_{e.s.H_2O}$) or the partial molecular volume (V) in the Eq.1 and to use the corrected values for predicting retention under other linear or multi-segment gradient runs. Retention times obtained experimentally from one initial experiment (0.0 min – 0%B, 60.0 min – 100%B) and structure data, were used to correct the ($\Delta G_{e.s.H_2O}$) and then to predict retentions of compounds for other gradient profile (0.0 min – 0%B, 30.0 min – 100%B). The experimental and predicted results for the second experiment are shown in the Fig. 1. and the deviations between experimental and simulated retention time values are not more than 4%.

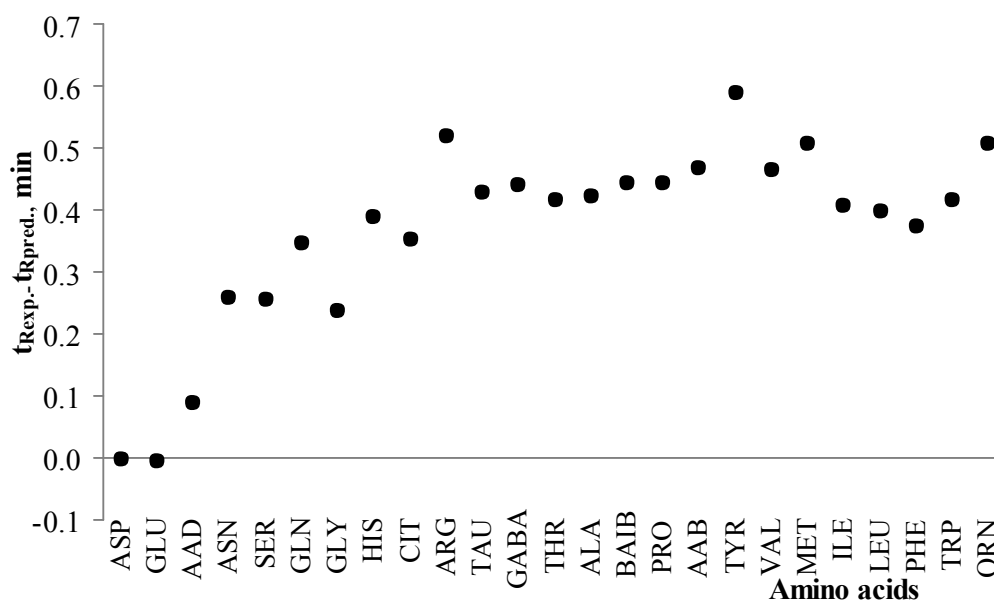


Fig. 1. Difference in retention times for predicted and experimental data.

Such deviations look as practically quite reasonable for prediction of retention to search for optimal gradient profile. Predicted elution order of the studied compounds corresponds to the experimental, resolution and retention time are practically identical to the experimental values with correlation coefficient of 0.998 (Fig.2).

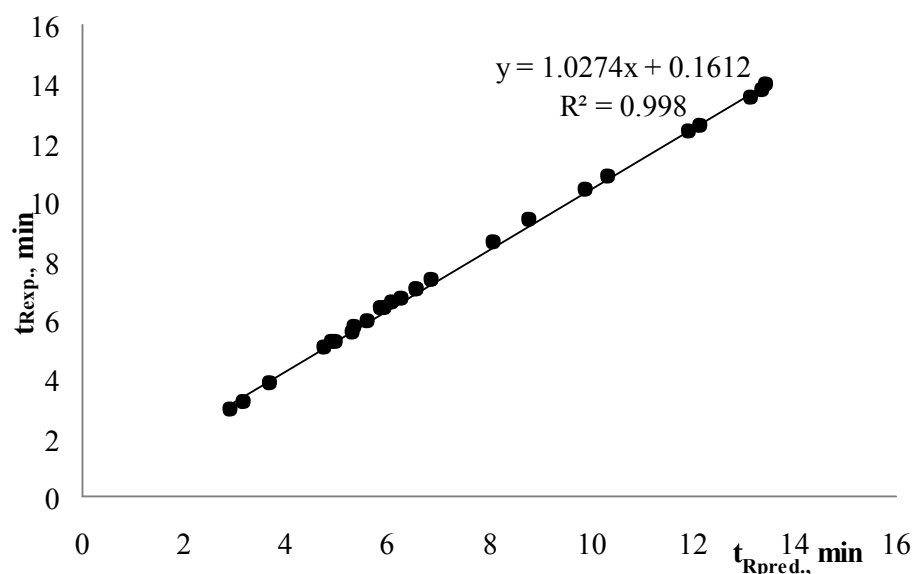


Fig. 2. Correlation of predicted and experimental retention time of amino acids from structure and one initial experiment data

The experiment retention times obtained experimentally from two initial experiments (0.0 min – 0%B, 60.0 min – 100%B and 0.0 min – 0%B, 45.0 min – 100%B), were used to fit both the value of energy of interaction ($\Delta G_{e.s.H_2O}$) and the partial molecular volume (V) in the Eq.1. and than used for prediction of retentions of compounds for another gradient profile (0.0 min – 0%B, 30.0 min – 100%B). The experimental and predicted results for the third experiment are shown in the Fig 3.

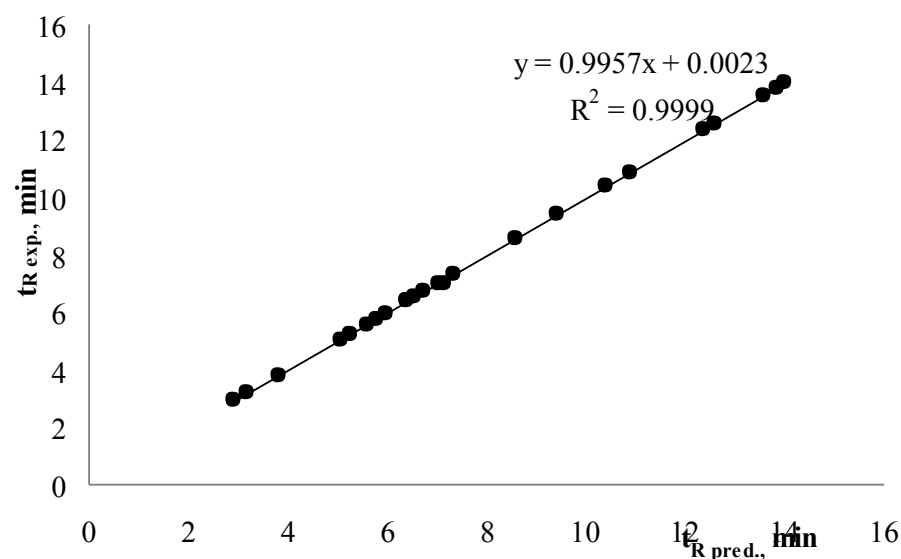


Fig. 3. Correlation of predicted and experimental retention time of amino acids from two experiment data

The solvatic model provides a good correlation between experimental and predicted retention times with the correlation coefficient of 0.9999 and can be an alternative to the linear retention model used usually in for computer-aided gradient optimization.

4. CONCLUSIONS

The solvatic model has been applied for prediction of retention of 25 phenylisothionate derivatives of amino acids in gradient mode of reversed-phase HPLC. The model enables prediction of

initial conditions from analyte chemical structure and column characteristics. The practically acceptable prediction of retention time values can be obtained after input of data of only one experiment. Data of two experiments as input enable precise prediction of retention both for the linear and solvatic retention models.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCE

1. Snyder LR, Kirkland JJ, Gajch JL. Practical HPLC method development. 2nd ed. New York, John Wiley&Sons; 1998.
2. Snyder LR, Dolan JW. High-Performance Gradient Elution–The Practical Application of the Linear-Solvent-Strength Model. NJ, Wiley–Interscience, John Wiley&Sons, Inc., Hoboken; 2007.
3. Kromidas S. HPLC Made to Measure. A Practical Handbook for Optimization. Weinheim, WILEY-VCH Verlag GmbH & Co; 2006.
4. Grushka E, Grinberg N. Advances in Chromatography 48. PressTaylor & Francis; 2009.
5. Put R, van der Heyden Y. Review on modelling aspects in reversed-phase liquid chromatographic quantitative structure–retention relationships. Anal. Chim. Acta. 2007;602:164-172.
6. Heberger K. Quantitative structure–(chromatographic) retention relationships. J. Chromatogr. 2007;1158:273-305.
7. Vitha M, Carr PW. The chemical interpretation and practice of linear solvation energy relationships in chromatography. J. Chromatogr. A. 2006;1126:143-194.
8. www.molnar-institute.com
9. www.chromsword.com
10. www.acdlab.com
11. Galushko SV. Calculation of retention and selectivity in reversed-phase liquid chromatography. J. Chromatogr. A. 1991;552:91-102.
12. Galushko SV, Kamenchuk AA, Pit GL. Calculation of retention in reversed-phase liquid chromatography: IV. ChromDream software for the selection of initial conditions and for simulating chromatographic behaviour. J. Chromatogr. A. 1994;660:47-59.
13. Galushko SV. The calculation of retention and selectivity in reversed-phase liquid chromatography II. Methanol-water eluents. Chromatographia. 1994;36:39-42.
14. Horváth C, Melander W, Molnár I. Solvophobic interactions in liquid chromatography with nonpolar stationary phases. J. Chromatogr. A. 1976;125:129-156.
15. Baczek T, Kaliszan R. Combination of linear solvent strength model and quantitative structure–retention relationships as a comprehensive procedure of approximate prediction of retention in gradient liquid chromatography. J. Chromatogr. A. 2002;962:41-55.
16. Baczek T, Kaliszan R. Predictive approaches to gradient retention based on analyte structural descriptors from calculation chemistry. J. Chromatogr. A. 2003;987:29-37.
17. Du H, Wang J, Yao X, Hu Z. Quantitative Structure-Retention Relationship Models for the Prediction of the Reversed-Phase HPLC Gradient Retention Based on the Heuristic Method and Support Vector Machine. J. Chromatogr. Sci. 2009;47:396-404.
18. Kaliszan R, Wiczling P, Markuszewski MJ, Al-Haj MA. Thermodynamic vs. extrathermodynamic modeling of chromatographic retention. J. Chromatogr. A. 2011;1218:5120-5230.
19. Hewitt EH, Lukulay P, Galushko S. Implementation of a rapid and automated high performance liquid chromatography method development strategy for pharmaceutical drug candidates. J. Chromatogr. A. 2006;1107:79-87.
20. Xiao KP, Xiong Y, Zhu Liu F, Rustum AM. Efficient method development strategy for challenging separation of pharmaceutical molecules using advanced chromatographic technologies. J. Chromatogr. A. 2007;1163:145-156.
21. Golusko J, Mekšs P, Shyshkina I, Galushko S. Prediction of Retention in Gradient Reversed – Phase Liquid Chromatography Using Chemical Structure and Column Characteristics. Latvian J. Chem. 2008;2:132-142.
22. Mengerink AY, Peters R, Wal Sj, van der Claessens HA, Cramers CA. Analysis of linear and cyclic oligomers in polyamide-6 without sample preparation by liquid chromatography using the sandwich injection method: III. Separation mechanism and gradient optimization. J. Chromatogr. A. 2002;949:307-326.

- 215 23. Nikitas P, Pappa-Louisi A. New approach to linear gradient elution used for optimisation in
216 reversed-phase liquid chromatography. J. Chromatogr. A. 2005;1068:279-287.
- 217 24. Nikitas P, Pappa-Louisi A, Agrafiotou P. Multilinear gradient elution optimisation in reversed-phase
218 liquid chromatography using genetic algorithms. J. Chromatogr. A. 2006;1120:299-307.
- 219 25. Nikitas P, Pappa-Louisi A, Papageorgiou A. Simple algorithms for fitting and optimisation for
220 multilinear gradient elution in reversed-phase liquid chromatography. J. Chromatogr. A.
221 2007;1157:178-186.
- 222 26. Pappa-Louisi A, Nikitas P, Papageorgiou A. Optimisation of multilinear gradient elutions in
223 reversed-phase liquid chromatography using ternary solvent mixtures. J. Chromatogr. A.
224 2007;1166:126-134.
- 225 27. Pappa-Louisi A, Agrafiotou P, Papachristos K. Retention modeling under organic modifier gradient
226 conditions in ion-pair reversed-phase chromatography. Application to the separation of a set of
227 underivatized amino acids. Anal. Bioanal. Chem. 2010;397:2151-2159.
- 228 28. Pappa-Louisi A, Agrafiotou P, Georgiadis I. Separation optimization in reversed-phase liquid
229 chromatography by using alkanol additives in the mobile phase: application to amino acids.
230 Talanta. 2011;85:2241-2245.
- 231 29. Hughes AB. Amino Acids, Peptides and Protein in Organic Chemistry. John Wiley&Sons Ltd; 2006.
- 232 30. Mant CT, Zhou NE, Hodges RS. Amino Acids and Peptides. J. Chromatogr. 1992;13:B76-B87.
- 233 31. Thomas MD. Textbook of Biochemistry with Clinical Correlations. 4th ed. John Wiley&Sons Ltd;
234 1997.