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

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ABSTRACT

Aim: To study the applicability of the solvatic retention model for the prediction of retention times in gradient elution of phenylisothiocyanate derivatives of natural amino acids

Methodology: 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 the structures of analytes and the characteristics of a column and an eluent using the ChromSword software package.

Results: The modeled initial estimation of retention time was found to be within 4% of the experimentally determined retention time. Fine-tuning of the model parameters using data from experimental runs further improved the accuracy of the model.

Conclusion: A 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 from successive runs can save time in method development and optimization of the 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 for organic compounds. Two modes of elution are used in HPLC: isocratic and gradient. Isocratic elution is suitable for the analysis of samples with a relatively narrow range of retention times and the gradient mode is used to separate compounds with a wide range of hydrophobicity and retention times. 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].

The relationship between retention time and the molecular structure of analytes has been an interesting and important theoretical and practical problem in chromatography for a long time. Prediction of retention using the chemical structures of compounds and column properties can save time and reduce the number of experiments required. 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 a few contain options to predict retention time from chemical structures. ChromSword uses the solvatic retention model [3, 11-13] which was derived from the solvophobic retention model of reversed-phase liquid chromatography (RP LC) [14]; and ACD ChromSimulator [3, 10] applies a linear relationship between the Log P value predicted from structural formulae and retention in reversed-phase liquid chromatography. Some articles have been published regarding the prediction of retention in gradient and isocratic elution from chemical structures [15-17] utilizing a generic equation: [retention] = $k_1 + k_2a_1 + \ldots + k_6a_5$, where ($a_1$-$a_5$) are different molecular descriptors and k1-k6 are coefficients to be determined from a so-called "training set" of compounds. Different software packages (ACD, HYPERCHEM, HEMPLUS, CLOGP, etc) have been used to calculate molecular descriptors [14-17]. This approach requires the 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 the prediction of retention times for other structures. Such 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 reversed phase chromatography and a detailed discussion of those approaches is not attempted in this article. In a recently published paper an attempt was made to apply the solvophobic model for prediction of retention from structural parameters [18]. However the approach was, in fact, described and applied substantially earlier [11-13] and realized in the commercial software [3, 10, 19, 20].

The solvatic model of retention to predict initial conditions in reversed phase liquid chromatography from chemical structure and column characteristics was also described earlier [11-13]. The model was used for the prediction of retention behavior of aromatic compounds [11-13], derivatives of triazine, phenylurea [21], plus linear and cyclic oligomers in polyamide-6 [22]. Those compounds are mainly neutral under normal conditions of chromatographic analysis and the applicability of the model has not been studied for highly polar and charged analytes. The aim of our work was to study the applicability of the solvatic retention model for the prediction of retention times 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 the capability to predict the retention time of these compounds from their chemical structure can potentially save time for method development and optimization of their separation. Although, several approaches to the prediction of retention of the amino acids and their derivatives have been previously studied [23-28] the algorithms for prediction of retention times described in this work are totally different. The main advantage of the proposed method is the feasibility of prediction of the initial gradient conditions without any previous experimental data, with reasonable results.

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

2. EXPERIMENTAL DETAILS

2.1 Equipment and software

Chromatographic measurements were made on an Alliance Waters 2695 liquid chromatographic system (Waters, Milford, MA, USA) consisting of Waters 2487 UV detector, temperature controlled column and autosampler (at 6ºC). A Waters Pico·Tag 30.0×0.39 cm I.D., particle size 4 µm column was employed as the stationary phase for all experiments. The injected sample volume was 10 µL. All chromatographic investigations were performed at 46ºC with an eluent flow-rate of 1 mL min^{-1}. Sample hydrolysis and derivatization were carried out with the Pico·Tag Workstation (Waters). Empower2 software (Waters, Milford, MA, USA) was used to acquire chromatographic data. For the prediction of retention parameters and resulting processing the 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 a Milli-Q water system (Millipore).

Each mobile phase composition was prepared separately. The first eluent was prepared as follows: 19.0 g sodium acetate trihydrate was dissolved in 1 L Milli–Q water, 0.5 mL TEA was added, mixed and titrated with glacial acetic acid to pH 6.40. The solution was filtered through a 0.45 µm filter. Finally 940 mL of the resulting solution was added to 60 mL acetonitrile (CH_3CN). The second eluent was prepared from CH_3CN and water in a ratio 3:2. The eluents were degassed by sonification.

Laboratory-prepared amino acids in 0.1 M HCl solution, were prepared from 25 commercially available amino acids (Sigma. For more convenient reading the abbreviations of the amino acids used 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 the amino acid sample was prepared by using phenylisothiocyanate derivatization and the elution pattern was recorded at 254nm [33-35].

3. RESULTS AND DISCUSSION

To calculate approximately the retention time, the solvatic retention model of reversed-phase chromatographic system was employed. The model has been described in detail [11-13] and an equation for calculating retention in reversed-phase chromatography and the 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 the 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, coefficient a = 16.48($\gamma_m - \gamma_s$), where γ_m and γ_s are the surface tension of a mobile and stationary phase correspondently, coefficient b= 0.8234×[f(ε_m) – f(ε_s)], where ε_m and ε_s are dielectric permittivity of a mobile and stationary phase correspondently and c is a parameter which includes the ratio of phases and some other characteristics of the stationary and mobile phases.

The approach of the computer-aided method development utilizes Equation 1 to calculate the retention in reversed-phase HPLC from chemical structure and column properties for prediction of initial gradient conditions (a first-guess linear gradient), and then fine-tuning the model after the input of data from successive experimental runs. Such a step-by-step method from the zero approximation (no run data available) to the first and second approximation (when data from 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 and 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 – the a, b, c coefficients in Equation 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 phenylisothiocyanate 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 following experiments was to predict the retention time much more precisely in order to start optimization of separation. Using the retention data obtained after the first linear gradient run, structure formula and column characteristics, it is possible to correct either the value of the energy of interaction ($\Delta G_{e.s.H_2O}$) or the partial molecular volume (*V*) in Equation 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 another gradient profile (0.0 min – 0%B, 30.0 min – 100%B). The experimental and predicted results for the second experiment are shown in Fig. 1; and the deviations between experimental and simulated retention time values are shown to be not more than 4%.

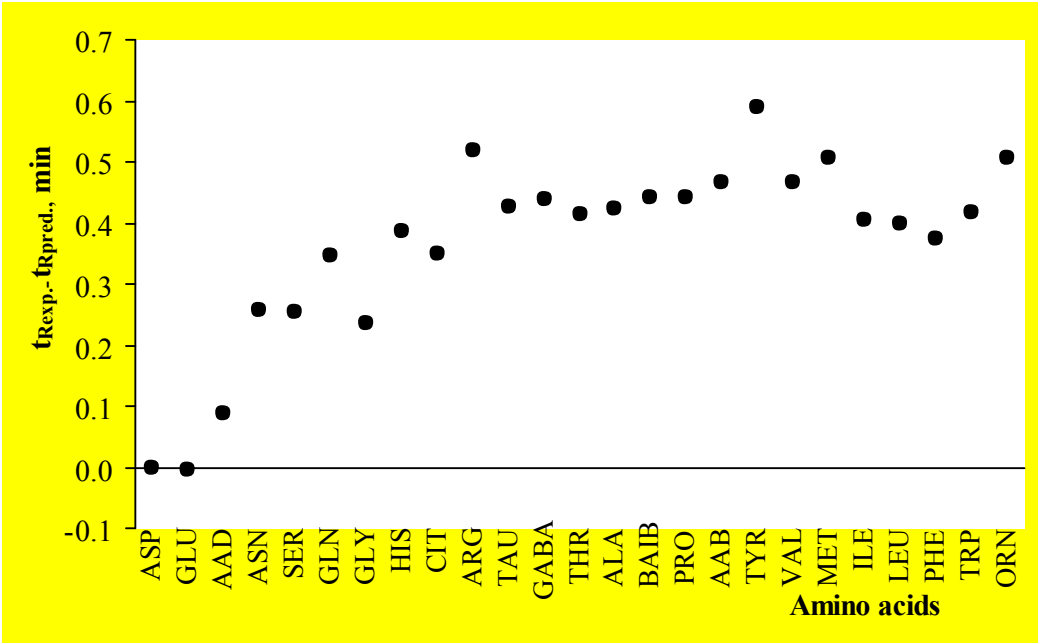

Fig. 1. Difference in retention times for predicted and experimental data.

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

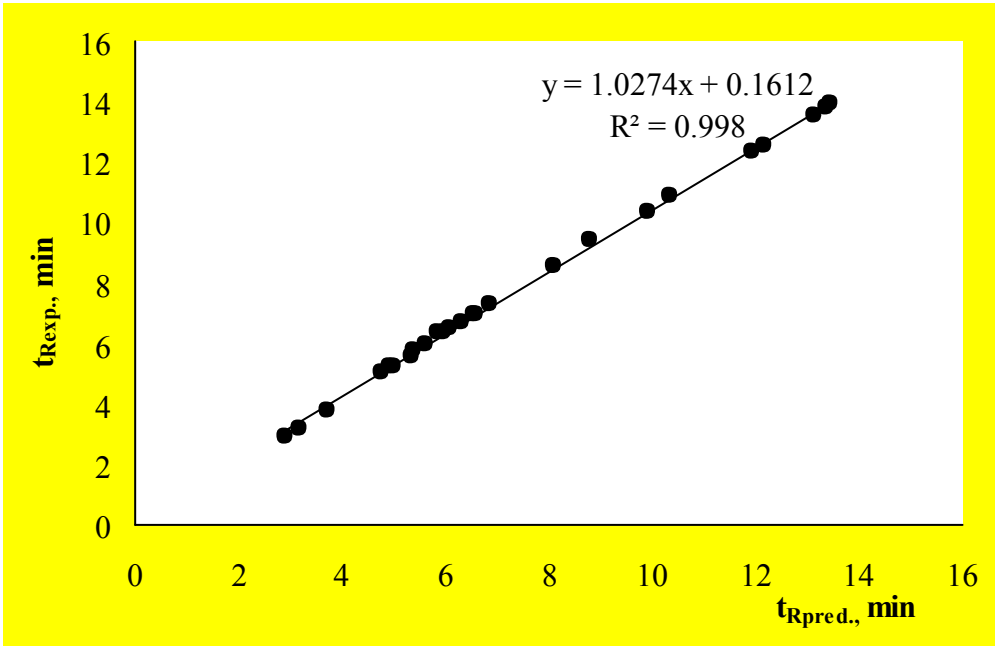

Fig. 2. Correlation of predicted and experimental retention time of amino acids from structure and one initial experiment data

The 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 were then used for the prediction of retention times 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 Fig. 3.

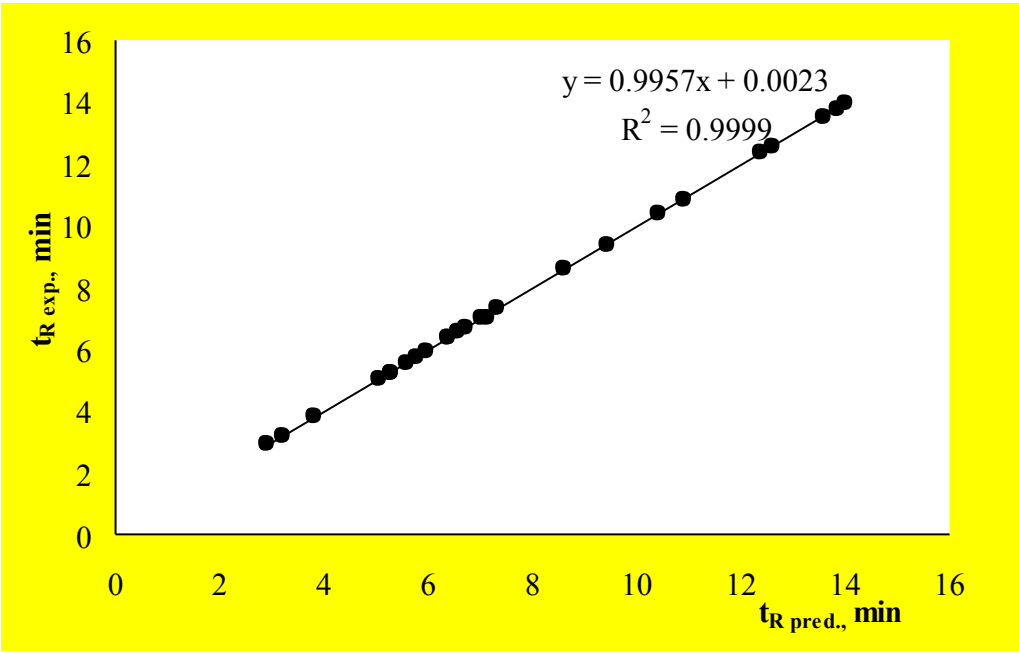

Fig. 3. Correlation of predicted and experimental retention time of amino acids using data from two experiments

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 often used for computer-aided gradient optimization.

4. CONCLUSIONS

The solvatic model has been applied for prediction of retention times of 25 phenylisothiocyanate derivatives of amino acids in the gradient mode of reversed-phase HPLC. The model enables the 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 from only one experiment. Data from two experiments as input enables the precise prediction of retention time both for the linear and solvatic retention models.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

AUTHORS` CONTRIBUTIONS

This work was carried out in collaboration between all authors. All authors declare an interest in the publication of the manuscript. The manuscript has not been published elsewhere and it has not been submitted simultaneously for publication in another journal.

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