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CHEMISTRY

Efficiency of a solvatic sorption model for the prediction of retention times in linear gradient reversed-phase liquid chromatography working with different stationary phases

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Abstract. Currently several different approaches are used for speed-up and cost reduction for new method development in reversed-phase high-performance liquid chromatography. During this research, application of a solvatic retention model of reversed-phase high-performance liquid chromatography was studied to predict the retention of phenylisothiocyanate derivatives of 25 natural amino acids, working with different stationary phases. The gradient elution mode was used, with methanol and acetonitrile as the aqueous mobile phases. Retention factors were calculated from the molecular parameters of the structures of the analytes and stationary and mobile phase properties. Such step-by-step methods, which include the first-guess prediction of initial conditions from structural formulae and fine tuning parameters of the retention model using data from successive runs, can save time and consequently will reduce the cost of method development and optimization.

Key words: high-performance liquid chromatography, solvation sorption model, stationary phases, ChromSword computer simulation software, phenylisothiocyanate derivatives of amino acids.

INTRODUCTION

In high-performance liquid chromatography (HPLC) reversed-phase liquid chromatography (RP-LC) is currently the separation mode of choice for the majority of analyses [1,2]. In many laboratories RP-HPLC has become an indispensable technique for the analysis of samples, allowing the determination of physical constants and the isolation of purified components from complex mixtures. RP-HPLC has found broad acceptance as the analytical technique of choice in many scientific and application-oriented areas such as food, environmental, pharmaceutical, and clinical chemistry; synthetic polymers; and various life sciences.

However, for more complex samples with more difficult separation requirements, enhanced methods are required. Two modes of elution are used in HPLC: isocratic and gradient. The isocratic elution mode, where the organic solvent in the mobile phase is not changed during the separation, is not suitable anymore. The gradient mode, where the composition of the mobile phase changes during the run, is used to separate compounds with a wide range of hydrophobicity and retention characteristics [3,4].

It is no secret that the development and optimization of HPLC gradient methods can be time consuming and require many experiments [3,4]. However, scientists can no longer afford to use the 'trial-and-error' approaches previously often applied.

In such areas as the pharmaceutical and clinical industries as well as food and beverages it is necessary to develop rapid optimization methods. So analysis time and financial considerations are also used as optimization criteria [5].

At present, rapid and adequate method development is one of the overriding issues in laboratory practice. During the last few years, several powerful software

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programs for method development and optimization have become available. Programs such as DryLab, ChromSword, and others have already been described in the literature [6–14]. However, the use of such programs for method development and optimization of analysis is still under study.

The solvatic retention model of RP-LC was described previously [15–18]. The model was applied to predict retention in isocratic elution from structural formulae of derivatives of 6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine (Lamotrigine), triazine, phenyl urea, and some aromatic compounds [15–17]. Recently we described the application of the solvatic model for the prediction of retention times of phenylisothiocyanate (PITC) derivatives of natural amino acids in gradient mode for linear gradient profiles working with the most popular C18 stationary phase [18]. During that study we obtained acceptably accurate predictions of retention time values.

The aim of our study was to check the efficiency of the solvatic sorption model for different stationary phases suitable for RP-HPLC. The efficiency of the solvatic sorbtion model was studied not only with different stationary phases, but also with different organic modifiers.

In this study we used MeOH and MeCN as organic modifiers. According to their polarity values (P'_{MeOH} = 5.1 and P'_{MeCN} = 5.8) and dielectric constant values (ε_{MeOH} = 32.7 and ε_{MeCN} = 37.5), the chosen organic modifiers have rather similar elution characteristics. However, MeOH and MeCN belong to different selectivity groups. The notion of partial polarities is discussed in the literature, and this is obtained by multiplying P′ by the proton acceptor power ($P'x_{eMeOH}$ = 2.448 and $P'x_{eMeCN}$ = 1.798), proton donor power ($P'x_{dMeOH}$ = 1.122 and $P'x_{dMeCN}$ = 1.566), and strength of dipole–dipole interaction ($P'x_{mMeOH}$ = 1.581 and $P'x_{mMeCN}$ = 2.436) [19–20]. Taking into account these values we can declare that the effect of MeOH can be mainly attributed to proton acceptor interaction, while that for MeCN corresponds to dipole–dipole interactions.

It is no less important to study how the model works for highly polar and charged analytes. For this reason we selected PITC derivatives of natural amino acids as research analytes. Derivatization with PITC provided an opportunity to use a simple chromatographic system because of pre-column derivatization. An additional bonus is that derivatives retain their stability for up to 48 hours (keeping samples at 5–8 °C) [21–24].

EXPERIMENTAL

Materials and supplies

Acetonitrile (ACN) and methanol (MeOH) of HPLC grade were obtained from LabScan. Analytically pure sodium acetate trihydrate, 6 M hydrochloric acid, and glacial acetic acid were obtained from Penta. Triethylamine (TEA) was obtained from Fluka and PITC was obtained from Sigma Aldrich, both HPLC grade. Water was purified by the Milli-Q water purification system (Millipore).

Two mobile phase solutions were prepared. For the experiments with ACN, the solution of the first eluent was prepared by dissolving 19.0 g sodium acetate trihydrate in 1 L Milli-Q water, adding 0.5 mL TEA, and adjusting the solution to pH 6.40 with glacial acetic acid. The prepared solution was filtered through a 0.45 μm filter. Finally 940 mL of the resulting solution was mixed with 60 mL ACN. The solution of the second eluent was prepared from ACN and water in a ratio 3 : 2. The eluents were degassed with sonification. For the experiments with MeOH, both eluents were prepared in the same way, but instead of ACN, MeOH was used.

Laboratory-prepared amino acids in 0.1 M HCl solution, composed of 25 commercially available amino acids (Sigma), were used. For more convenient reading the abbreviation and structure of the used PITC derivatives of amino acids are given in Table 1.

The amino acid sample for the experimental work was prepared by pre-column PITC derivatization [24].

Equipment and software

The liquid chromatographic system Alliance Waters 2695 (Waters, Milford, MA, USA) consisted of a Waters 2487 UV detector and autosampler (at 6 °C) and a column oven. Sample hydrolysis and derivatization were carried out with a Pico·Tag Workstation (Waters).

To acquire chromatographic data, Empower 2 software (Waters, Milford, MA, USA) was used. The ChromSword computer simulation system, version 4.8.3.2010 (ChromSword, Germany), was used for the retention parameter prediction and result processing.

An OAKTON (Oakton Instruments, Vernon Hills, IL, USA) pH meter equipped with a glass electrode was used for pH measurements. The electrode was calibrated daily with appropriate buffers.

The examined RP-HPLC columns are reported in Table 2. Column temperature was maintained at 46 °C. A flow rate of 1 mL/min was used and 10 μL of the sample was injected. The detection of the analytes was performed at 254 nm.

Table 1. Composition of the 25 phenylisothiocyanate derivatives of the amino acid mixture used

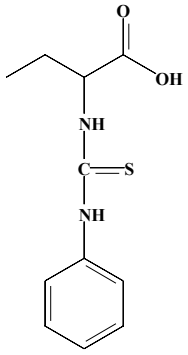
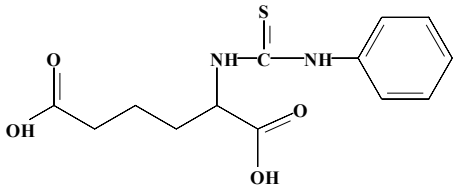
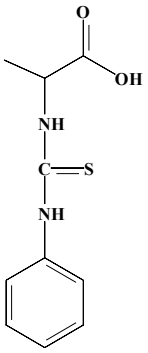
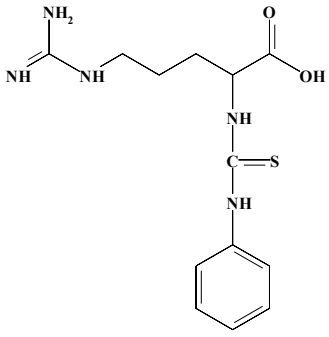
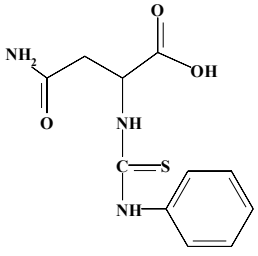
No.	Amino acid	Abbreviation*	Structure of PITC derivatives of amino acids
1	α-Aminobutyric acid	Aab	
2	α-Aminoadipic acid	Aad	
3	Alanine	Ala	
4	Arginine	Arg	
5	Asparagine	Asn	

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Table 1. *Continued*

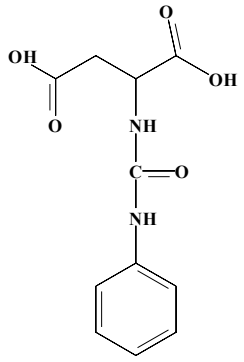
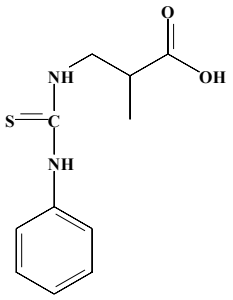
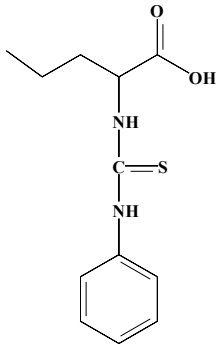
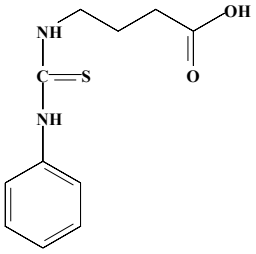
No.	Amino acid	Abbreviation*	Structure of PITC derivatives of amino acids
6	Aspartic acid	Asp	
7	β-Aminoisobutyric acid	Baib	
8	Citrulline	Cit	
9	γ-Amino butyric acid	Gaba	

Table 1. *Continued*

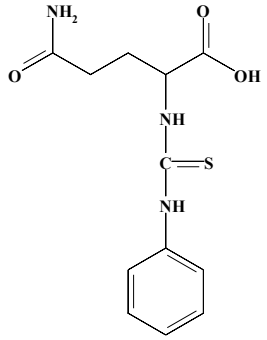
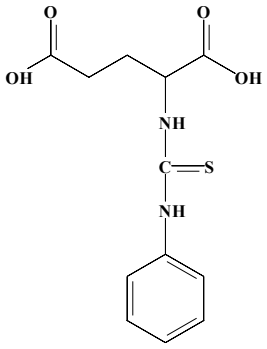
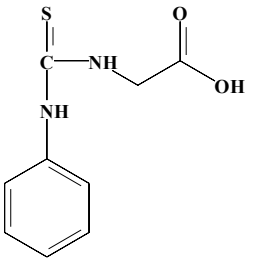
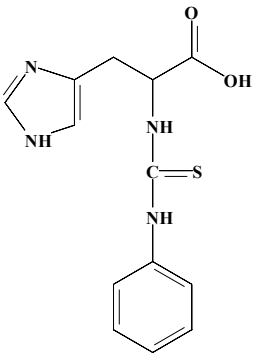
No.	Amino acid	Abbreviation*	Structure of PITC derivatives of amino acids
10	Glutamine	Gln	
11	Glutamic acid	Glu	
12	Glycine	Gly	
13	Histidine	His	

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Table 1. *Continued*

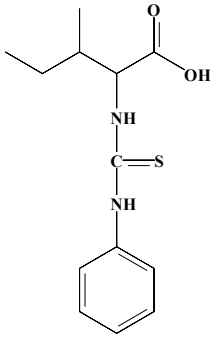
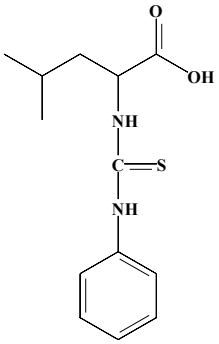
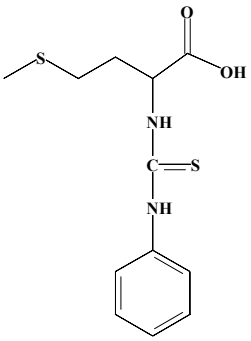
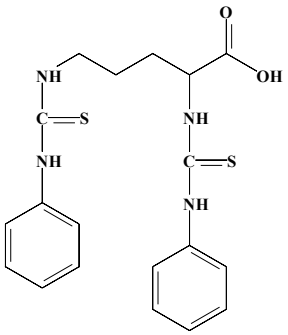
No.	Amino acid	Abbreviation*	Structure of PITC derivatives of amino acids
14	Isoleucine	Ile	
15	Leucine	Leu	
16	Methionine	Met	
17	Ornithine	Orn	

Table 1. *Continued*

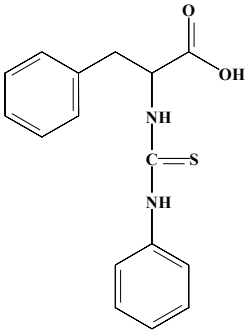
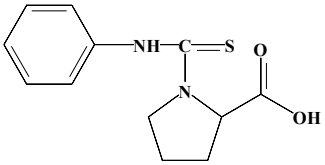
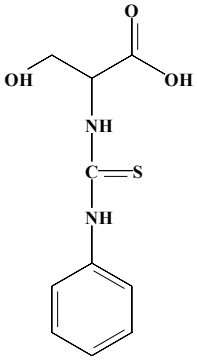
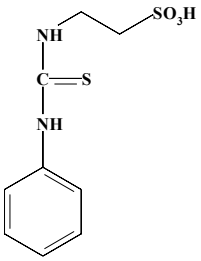
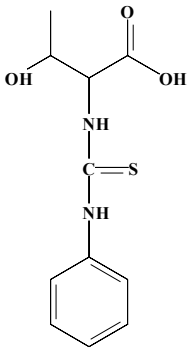
No.	Amino acid	Abbreviation*	Structure of PITC derivatives of amino acids
18	Phenylalanine	Phe	
19	Proline	Pro	
20	Serine	Ser	
21	Taurine	Tau	
22	Threonine	Thr	

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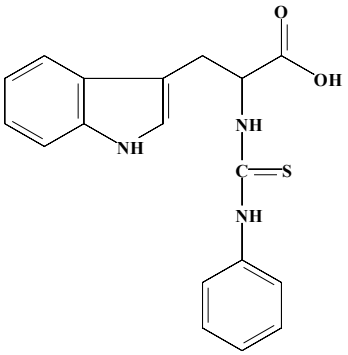
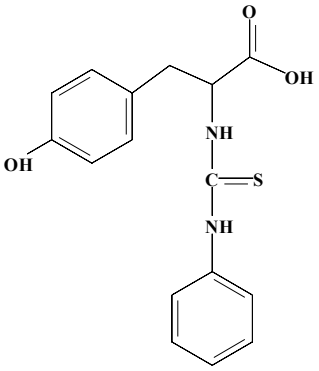
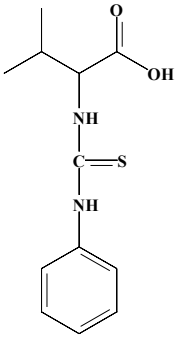
Table 1. *Continued*

No.	Amino acid	Abbreviation*	Structure of PITC derivatives of amino acids
23	Tryptophane	Trp	
24	Tyrosine	Tyr	
25	Valine	Val	

* Abbreviations according to IUPAC recommendations.

Table 2. Investigated RP-HPLC columns (internal diameter of all columns 4.6 mm, particle size 5 μm)

Column	Stationary phase	Length, mm	Pore size, Å	Vendor
SunFire C18	Silica/C18	250	100	Waters, USA
Zorbax CN	Silica/CN	250	60	Agilent Technologies, USA
Zorbax SB-C8	Silica/C8	150	80	Agilent Technologies, USA
Nucleosil 100 C8	Silica/C8	250	100	MACHEREY-NAGEL GmbH & Co, Germany
Alltima C8	Silica/C8	250	100	GRACE, USA
YMC-Pack C4	Silica/C4	150	300	YMC, USA
Mixed Mode RP-C18/Cation	Polymer/C18/Cation	250	100	GRACE, USA
Mixed Mode RP-C8/Cation	Polymer /C8/Cation	250	100	GRACE, USA

RESULTS AND DISCUSSION

For evaluating the efficiency of the solvatic model, 25 PITC derivatives of natural amino acids (Table 1) were used in the present study. To calculate the retention time, the solvatic retention model of the reversed-phase chromatographic system was employed. The model was described in detail in [13,14,25] and an equation for calculating the retention time in reversed-phase chromatography and the calibration of columns was derived:

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

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

Two types of experiments were carried out to verify the model in this study. One (zero approximation) was directed at the prediction of the initial gradient conditions of retention in the RP-HPLC from the chemical structure of analytes (Table 1) and column properties. The method requires that both parameters of the solutes (the volume and energy of the interaction with water) and the characteristics of the reversed-phase column under experimental conditions should be known. The main idea of this type of experiments in computer-assisted method development is to provide practically reasonable retention time values for target compounds. For this purpose we used the commercially available ChromSword software [7]. This software contains a database of column characteristics of many commercially available reversed-phase columns: the coefficients *a*, *b*, and *c* in Eq. (1) at any concentration of acetonitrile and methanol in the mobile phase.

Using all the necessary parameters for zero approximation, we predicted the retention time for the linear gradient (0.0 min – 0%B, 60.0 min – 100%B) for all columns working with both organic modifiers. For data comparison we experimentally obtained retention times of analytes following a linear gradient run.

The difference in the retention times for predicted and experimental data using zero approximation are shown in Fig. 1 for acetonitrile as the organic modifier and in Fig. 2 for methanol as the organic modifier. We observed that the prediction of the retention times was not satisfactory; however, the predicted elution order of the studied compounds predominantly corresponded to the experimentally obtained elution order. Moreover, from this experiment we know the required concentration of the organic modifier for the elution of all constituents of the mixture. Based on the data obtained from this experiment, we were making progress to achieve practically acceptable prediction of retention time values.

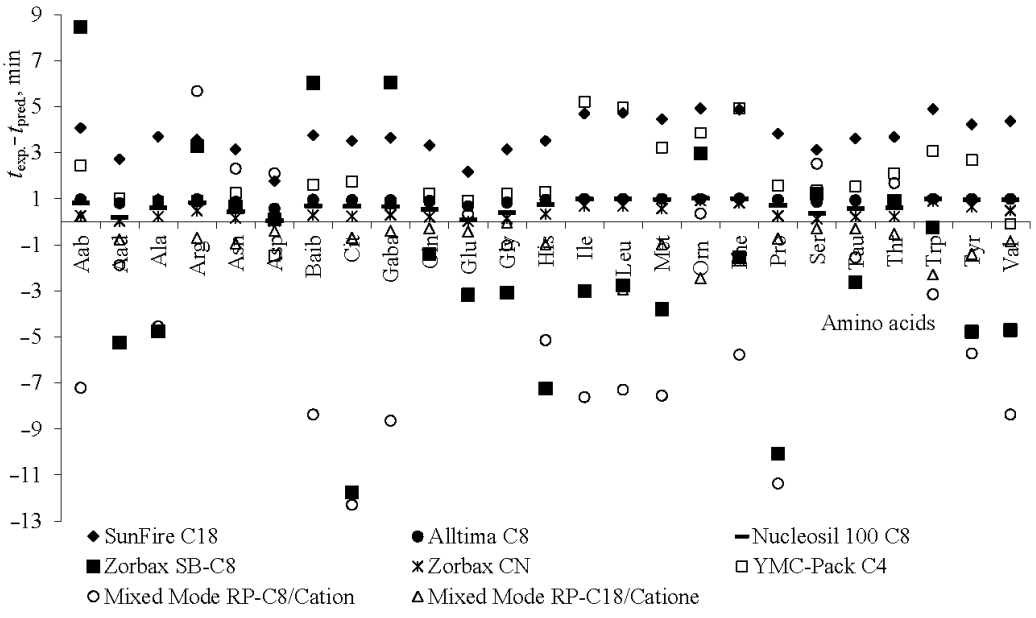


Fig. 1. Difference in the retention times for predicted and experimental data using acetonitrile as the organic modifier.

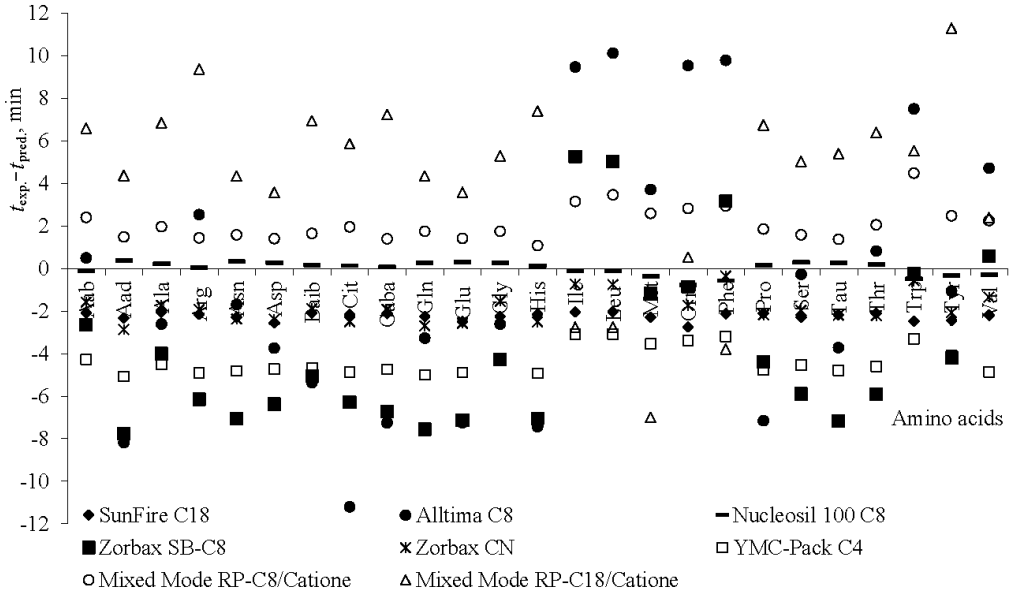


Fig. 2. Difference in the retention times for predicted and experimental data using methanol as the organic modified.

The other of experiment (first approximation and second approximation) involves the prediction from structural formulae of the analytes and the characteristics of the reversed-phase column and results of one experimental run for the first approximation and of two runs for the second approximation. The goal of the experiments was to predict the retention time much more precisely 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 ($\Delta G_{e.s.\mathrm{H_2O}}$) or the partial molecular volume (V) in Eq. (1) and to use the corrected values for predicting retention under other linear or multi-segment gradient runs.

We corrected the ($\Delta G_{e.s.\mathrm{H_2O}}$) drawing on the retention data experimentally obtained after the first linear gradient run (0.0 min – 0%B, 60.0 min – 100%B) and the structure data. Then we predicted the retention times of compounds for other gradient profiles (0.0 min – 0%B, 45.0 min – 100%B).

The predicted elution order of the studied analytes corresponds to the experimental results. The agreement between the predicted retention times and experimental data is confirmed by correlation analysis (Table 3 for acetonitrile–aqua and methanol–aqua mobile phases). The solvatic model provides a good correlation between experimental and predicted retention times with the correlation coefficient not less than 0.9991 for the SunFire C18 column in the acetonitrile–aqua mobile phase and not less than 0.998 for the Mixed Mode RP-C18/Cation column in the methanol–aqua mobile phase. The correlation coefficients strongly support the validity of the approach.

The retention times obtained experimentally from the 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 the energy of interaction ($\Delta G_{e.s.\mathrm{H_2O}}$) and the partial molecular volume (V) in Eq. (1). After that these retention times were used for the prediction of retention times of compounds for another gradient profile (0.0 min – 0%B, 30.0 min – 100%B).

To illustrate the gradient retention prediction capabilities of the solvatic model, the experimentally obtained data were plotted against the predicted ones. The results are presented in Table 4 for both organic modifiers. Analysis of the data obtained on different columns showed broader scattering of data points for the Mixed Mode RP-C8/Cation column and Mixed Mode RP-C18/Cation column than for the other columns (Mixed Mode RP-C8/Cation column R^2 = 0.9985 and $R^2 = 0.998$, Mixed Mode RP-C18/Cation column $R^2 = 0.9988$ and $R^2 = 0.998$ for acetonitrile-containing eluent and methanol-containing eluent, respectively). The reason may be the reversed-phase and cation-exchange mechanism for these columns. In mixed-mode columns, significant retention occurs via the cation-exchange sites of the stationary phase. In spite of this, these columns were used in pure reversed-phase mode for the separation of neutral hydrophobic compounds.

Table 3. Correlation of predicted and experimental retention times of phenylisothiocyanate derivatives of amino acids from the structure and one initial experiment data (0.0 min – 0%B, 60.0 min – 100%B) for acetonitrile–aqua and methanol–aqua mobile phases

Sorbent	ACN		MeOH	
	Correlation coefficients (R^2)	Regression equation	Correlation coefficients (R^2)	Regression equation
SunFire C18	0.9991	$y = 0.9804x + 1.8082$	0.9997	$y = 1.0139x + 0.616$
Zorbax CN	0.9998	$y = 1.076x – 0.2834$	0.99990	$y = 0.9419x + 0.2076$
Zorbax SB-C8	0.9998	$y = 1.0241x – 0.0334$	0.9992	$y = 1.02x + 0.255$
Nucleosil 100 C8	0.99999	$y = 1.7409x – 0.0026$	0.9993	$y = 1.0542x – 0.0046$
Alltima C8	0.9992	$y = 1.0241x + 0.4218$	0.9995	$y = 1.0201x + 0.6921$
YMC-Pack C4	0.99990	$y = 1.0873x – 0.2313$	0.99990	$y = 1.1328x – 0.389$
Mixed Mode RP-C18/Cation	0.99990	$y = 1.0675x – 0.1774$	0.9996	$y = 1.0584x – 0.0181$
Mixed Mode RP-C8/Cation	0.9992	$y = 1.14x – 0.4249$	0.998	$y = 0.8771x + 0.064$

Table 4. Correlation of predicted and experimental retention times of phenylisothiocyanate derivatives of amino acids from the structure and data of two initial experiments (0.0 min – 0%B, 60.0 min – 100%B and 0.0 min – 0%B, 45.0 min – 100%B) for acetonitrile–aqua and methanol–aqua mobile phases

Sorbent	ACN		MeOH	
	Correlation coefficients (R^2)	Regression equation	Correlation coefficients (R^2)	Regression equation
SunFire C18	0.99990	$y = 1.0088x – 0.0294$	0.99999	$y = 1.0036x – 0.008$
Zorbax CN	0.9998	$y = 0.9837$x+0.103	0.9997	$y = 0.9995$x+0.0124
Zorbax SB-C8	0.99990	$y = 1.0047x – 0.0031$	0.9986	$y = 1.0241x – 0.1635$
Nucleosil 100 C8	0.99999	$y = 0.9969$x+0.0205	0.9987	$y = 1.0392x – 0.2623$
Alltima C8	0.9997	$y = 1.0113x – 0.051$	0.9993	$y = 1.0038$x+0.0733
YMC-Pack C4	0.99990	$y = 1.0006$x+0.0099	0.99999	$y = 1.0001$x+0.0082
Mixed Mode RP-C18/Cation	0.9988	$y = 1.0063$x+0.1028	0.998	$y = 1.0206x – 0.028$
Mixed Mode RP-C8/Cation	0.9985	$y = 0.997$x+0.0744	0.998	$y = 1.0185x – 0.058$

The solvatic model provides a good correlation between experimental and predicted retention times and can be an alternative to the linear retention model often used for computer-aided gradient optimization. Student's *t*-test for the regression equation constant was performed for all stationary phases in acetonitrile–aqua and methanol–aqua mobile phases. We can claim that the slope of the equation was not statistically significantly different from one and the intercept was not significantly different from zero. So we conclude that there is a significant relationship between the experimentally obtained retention times and predicted retention times. Therefore, the retention prediction obtained by the chosen approach is very satisfactory: the percentage error of the predicted values is very low, lower than 8.3% (Table 5).

CONCLUSIONS

The efficiency of a solvatic sorbtion model for the prediction of the retention times of 25 PITC derivatives of amino acids in the gradient mode of RP-HPLC for different stationary phases was studied. Evaluation of retention in terms of the chemical structure of the analytes and the physico-chemical properties of both the mobile and stationary phases was used for the prediction of initial conditions. A practically acceptable prediction of retention time values can be obtained after the input of data from only one experiment. Data from two experiments as input enabled a precise prediction of the retention time both for the linear and solvatic retention models. The described method of the prediction of retention time can substantially reduce the time required to find optimal conditions in gradient elution.

Table 5. Percentage error of the predicted values for the gradient profile (0.0 min – 0%B, 30.0 min – 100%B)

Compound*	SunFire C18		Alltima C8		Nucleosil 100 C8		Zorbax SB-C8		Zorbax CN		YMC-Pack C4		Mixed Mode RP-C8/Cation		Mixed Mode RP-C18/Cation	
	MeOH, %	ACN, %	MeOH, %	ACN, %	MeOH, %	ACN, %	MeOH, %	ACN, %	MeOH, %	ACN, %	MeOH, %	ACN, %	MeOH, %	ACN, %	MeOH, %	ACN, %
Aab	0.4	0.8	0.7	0.9	0.6	0.1	0.6	0.6	0.0	0.5	0.1	0.4	0.4	0.5	2.8	2.1
Aad	0.2	0.4	0.2	0.0	0.2	0.0	0.5	0.6	5.8	1.8	0.2	0.0	2.7	0.3	1.1	8.3
Ala	0.4	0.8	0.6	1.5	0.1	0.0	6.6	0.1	0.8	1.2	0.5	0.1	0.5	0.2	1.2	0.9
Arg	0.9	0.7	1.4	1.7	0.6	0.0	0.6	0.5	0.4	0.3	0.3	0.4	0.3	0.5	2.7	1.8
Asn	0.4	0.4	0.4	0.1	0.6	0.0	0.4	0.0	0.5	0.9	0.5	0.1	1.2	0.2	1.1	2.4
Asp	0.0	0.9	0.2	0.4	0.4	0.0	0.6	0.1	0.4	0.4	0.2	0.4	2.1	0.3	0.1	8.3
Baib	0.6	0.8	0.7	0.6	0.9	0.0	0.6	0.5	0.4	1.2	0.3	0.4	0.7	0.2	1.2	0.9
Cit	0.5	0.5	0.8	3.6	3.1	0.0	0.1	0.2	0.6	0.9	0.2	0.7	0.4	0.3	4.0	0.9
Gaba	0.4	0.1	0.7	0.3	0.7	0.0	0.5	0.1	0.4	1.2	0.5	0.1	0.5	0.7	1.2	0.9
Gln	0.2	0.4	0.5	0.2	0.4	0.0	0.4	0.1	0.5	0.8	0.5	0.9	0.6	0.2	1.1	0.9
Glu	0.0	0.1	0.2	0.0	0.3	0.0	0.1	0.1	0.4	0.4	0.0	0.1	2.1	0.3	0.1	1.6
Gly	0.4	1.3	0.5	0.1	0.2	0.0	0.5	0.4	0.8	0.8	0.5	0.9	1.1	0.2	0.7	0.9
His	1.2	2.0	0.6	0.5	0.4	0.0	0.9	0.5	5.7	0.5	0.3	0.4	0.1	0.2	1.2	0.9
Ile	0.0	0.6	0.4	0.1	4.9	0.2	0.3	0.5	0.1	0.8	0.1	0.5	6.8	0.5	2.1	1.0
Leu	0.4	0.7	0.9	1.0	4.9	0.1	0.3	0.5	0.1	0.8	0.1	0.4	0.7	1.0	2.1	1.0
Met	0.3	0.8	0.6	1.0	0.8	0.2	0.5	0.4	0.6	1.1	0.2	0.4	0.5	0.5	4.0	1.8
Orn	0.4	0.8	0.6	1.0	1.1	0.2	4.4	0.5	0.2	0.4	0.1	0.6	1.0	1.2	2.1	2.1
Phe	0.4	0.7	0.4	0.9	1.1	0.0	0.5	0.5	0.1	0.5	0.1	1.1	0.9	1.0	0.7	1.1
Pro	0.6	0.7	0.9	0.6	0.9	0.0	0.1	0.2	2.3	1.2	0.2	0.7	0.5	0.2	1.2	1.8
Ser	0.2	0.4	0.5	0.2	0.6	0.0	0.8	2.6	0.5	0.9	0.2	0.9	0.6	0.2	2.4	2.4
Tau	0.3	0.4	0.2	0.4	0.4	0.0	1.9	0.2	0.6	0.9	0.2	0.6	0.9	0.2	0.7	0.9
Thr	0.5	0.7	2.7	0.3	0.4	0.0	0.5	0.5	0.8	0.9	0.1	0.3	0.4	0.3	1.5	0.9
Trp	0.4	0.7	0.5	1.0	2.5	0.2	4.4	0.1	0.1	0.3	0.1	0.6	0.2	3.9	2.1	1.9
Tyr	0.1	0.8	0.7	1.1	0.8	0.1	0.5	0.4	0.0	1.1	0.0	0.1	0.7	0.9	2.1	1.8
Val	0.3	0.8	0.7	1.1	0.8	0.1	0.9	0.3	0.5	1.0	0.2	0.7	0.5	0.1	2.1	1.8

* See Table 1 for abbreviations.

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Solvaatsorptsiooni mudeli efektiivsus retentsiooniaegade ennustamisel lineaarse gradiendiga pöördfaas-vedelikkromatograafias erinevate statsionaarsete faaside korral

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Tänapäeval kasutatakse pöördfaas-kõrgefektiivses vedelikkromatograafias uute meetodite arendamise kiirendamiseks ja kulude vähendamiseks erinevaid lähenemisteid. Käesolevas uuringus kasutati pöördfaas-kõrgefektiivse vedelikkromatograafia solvaatretentsiooni mudelit 25 fenüülisotiotsüanaadi loodusliku aminohappe derivaadi retentsiooniaegade prognoosimiseks erinevate statsionaarsete faaside puhul. Kasutati gradientelueerimist metanool-vesi- ja atsetonitriil-vesi-mobiilsetes faasides. Retentsioonifaktorid arvutati analüütide molekulaarsete struktuuriparameetrite ja statsionaarse ning mobiilse faasi parameetrite alusel. Sellised järkjärgulised meetodid, mis sisaldavad lähtetingimuste esmast prognoosimist ainete struktuurvalemite alusel ja protsessi edasist peenhäälestamist järjestikuste kromatografeerimiste parameetrite põhjal, säästavad aega ning järelikult alandavad meetodi arendamise ja optimeerimise kulusid.