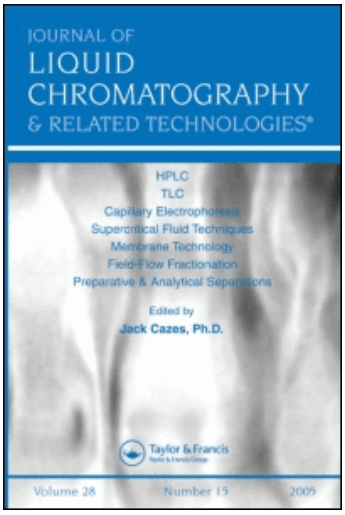

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Predictions of Peptide Retention in HPLC with the use of Amino Acid Retention Data Obtained in a TLC System

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Abstract: A thin-layer chromatography (TLC) experiment was used to find a new structural descriptor based on empirical data, which can be useful for prediction of high performance liquid chromatography (HPLC) retention of peptides. The optimization of TLC separation of a series of twenty naturally occurring amino acids was performed and, finally, the mobile phase comprising water and ethanol 95° (20:80 *v*/*v*) appeared to be an optimal one. The designed TLC experiment enabled obtaining different values of retardation factor, R_f, and to further calculate R_M values for individual amino acids. The sum of calculated R_M values, corresponding to the individual amino acids in the appropriate peptide, and four other descriptors calculated from the peptides' structural formulas using molecular modeling methods, were used in quantitative structure retention relationships (QSRR) analysis to predict retention times of a series of structurally diversified peptides chromatographed in a reversed phase HPLC system.

Keywords: Peptides, Amino acids, Thin layer chromatography (TLC), High performance liquid chromatography, Quantitative structure retention relationships (QSRR), Proteomics

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INTRODUCTION

Proteomics is the global analysis of complex protein mixtures for the purpose of qualitative, quantitative, and functional analysis of all the proteins present in a sample. Separation of the complex mixtures of peptides resulting from the total digest of protein is the initial step of analysis in the so called bottom up approach.[1] Optimization of chromatographic separations of peptides provides the means to obtain a complete resolution and identification of all analytes in a minimal time range. It is well known that chromatographic retention time of any peptide is strongly dependent on its chemical structure (e.g., amino acids composition, peptide chain length, peptide sequence), as well as on the chromatographic system conditions (e.g., mobile phase composition, stationary phase, temperature, pH). Although the prediction of peptides' retention cannot be considered just only on the basis of retention of amino acids, their individual retention contribution is crucial for that analysis and might be used to improve the confidence of peptide identifications and increase the number of correctly identified peptides.[2,3]

Several, already published reports, demonstrate that chromatographic behavior of peptides is strictly associated with their amino acids composition.[3–7] In 1980, Meek[4] assumed that the retention time of a peptide could be predicted using the sum of retention coefficients that represent the contribution to retention of each of the common amino acids. It was demonstrated that retention coefficients can be obtained directly from HPLC data for all amino acids, such that retention time, t_R, of peptide could be predicted from the sum of retention coefficients for each amino acid. A similar strategy was demonstrated by Brown et al.[3] Casal et al.[5] and Guo et al.[6] but with different values of retention coefficients of the same amino acids in different peptides. Mant et al.[7] additionally considered the polypeptide chain length along with the contribution of amino acids to the retention of peptides. In 2005, Wang et al.[8] demonstrated that for large peptides its reversed phase chromatographic behavior can be corrected with their amino acid composition, the number of residues in a polypeptide chain, and the ratio of contacting surface area of a peptide with the stationary phase.

Evaluation of retention in terms of chemical structure of analytes and of physicochemical properties of both the mobile and stationary phase is known under the acronym QSRR – quantitative structure retention relationships. QSRR are statistically derived relationships between chromatographic parameters and the quantities (descriptors) characterizing the molecular structure of analytes.[9–11] Chromatography appears to be an unique and highly suitable system to search the interaction between physicochemical properties of analytes and their molecular structure. This is because, in the chromatographic system, all the measurement conditions can be kept constant for a large, statistically representative series of structurally diverse analytes.

Chromatographic retention time is a chemical structure dependent parameter, which is constant for given separation conditions.[11–13] A good

prediction of gradient HPLC retention times of peptides was recently obtained by means of a QSRR model employing the following analyte descriptors:

(i) logarithm of the sum of gradient retention times of amino acids building the individual peptide, log Sum_{AA}
(ii) logarithm of the peptide's van der Waals volume, log VDW_{Vol}
(iii) logarithm of its theoretically calculated *n*-octanol/water partition coefficient, *clog P*.[14,15]

The retention parameter used recently in QSRR studies and derived from the TLC experiment is the R_M values [$R_M = \log (1/R_f - 1)$] where R_f is the ratio of a distance passed by the analyte to that attained by the solvent front.[16] However, previously the QSRR analysis was used for the studies on the predictions of peptides' retention in the TLC systems with the addition of ionic liquid into the mobile phase.[17] A reasonable, good agreement ($R = 0.91$) between the R_M values received experimentally and calculated with the use of the proposed QSRR model was obtained for a set of structurally diversified peptides, and proved the ability of QSRR to be a useful tool for the preliminary explanation of the peptide retention behavior in TLC systems.

A wide application of TLC in amino acids, protein, and peptide analysis for amino acid and peptide mapping, structural analysis, and identification of peptides, protein, and peptide fractionation, as well as determination of molecular weights of peptides and proteins,[2] was found. The aim of the work was to find new descriptors, which could be used for predictions of peptides' retention in HPLC using data obtained from the TLC experiment. The first step was to find the appropriate mobile phase composition that could make possible the separation of amino acids and, further, get the values of descriptors that allow for prediction of peptides' retention in HPLC. Amino acids' retention in TLC systems with different eluents was, therefore, studied. Finally, quantitative structure retention relationships (QSRR) were used for the prediction of peptides' retention in an HPLC system using TLC data.

EXPERIMENTAL

Materials

The following amino acids: alanine (A), arginine (R), asparagines (N), aspartic acid (D), cysteine (C), glutamic acid (E), glutamine (Q), glycine (G), histidine (H), isoleucine (I), leucine (L), lysine (K), methionine (M), phenylalanine (F), proline (P), serine (S), threonine (T), tryptophan (W), tyrosine (Y), valine (V), were from Fluka (Buchs, Switzerland). The following peptides were purchased from Sigma (St. Louis, MO, USA): AA,

AG, YL, GL, WG, AF, and GHG. Other peptides used in the study were synthesized at the Department of Organic Chemistry, University of Gdańsk.[14] Water was prepared with a Milli-Q Water Purification System (Millipore Corporation, Bedford, MA, USA). Trifluoroacetic acid (TFA) was provided by Fluka (Buchs, Switzerland). Other reagents used were as follows: hydrochloric acid, formic acid, acetic acid, methanol, ethanol, 2-propanol, 1-buthanol, acetonitrile (ACN), ammonium, chloroform, all provided by P.O.Ch (Gliwice, Poland).

Thin-Layer Chromatography Experiments

In the TLC experiment, aluminum backed plates covered with silica gel (NP-TLC 60 F_{254} and RP-18 F_{254S}) were used. The ready made TLC plates were from Merck (Darmstadt, Germany). Ninhydrin (Fluka, Buchs, Switzerland) was used as a label.

Five μL sample volumes were applied on TLC plates with glass micropipettes. Plates were developed in vertical chambers saturated with gas phase solvent for 20 minutes prior to the analysis. Developed plates were dried in air at room temperature and sprayed with ninhidrine solution to determine amino acid spots. After that, plates were dried in temperature of 60°C for 15 min.

Twenty amino acids were grouped into 5 subgroups (1 – N, T, M, W; 2 – C, Q, V, L; 3 – H, P, E, F; 4 – R, S, D, Y; 5 – K, G, A, I). Amino acids' solutions were prepared in the mixture comprised of acetic acid, ethanol 95°, and deionized water (2:1:1 *v/v/v*). Each determination was performed at ambient temperature (20 ± 2°C). The retention data were reported as average values of five independent experiments.

HPLC Experiments

Chromatographic measurements were performed with the use of an HPLC apparatus, LC Module I plus from Waters (Milford, MA, USA), equipped with a pump, variable wavelength UV/VIS detector, autosampler, and thermostat (Model Code LCH). Data were collected using Waters Millenium 2.15 software. XTerra MS C18 column (15.0 × 0.46 cm I.D., particle size 5 μm) from Waters (Milford, MA, USA) packed with octadecyl-bonded silica, was used in the study. Gradient HPLC elution was carried out with solvent A (water with 0.12% TFA), solvent B (ACN with 0.10% TFA). The mobile phase used was filtered through a GF/F glass microfibre filter (Whatman, Maidstone, UK) and degassed with helium during the analysis. The gradient was formed from 0% to 60% B within 20 min. All the chromatographic measurements were done at 40°C with an eluent flow rate of 1 mL/min. The experiments were performed at a detection wavelength of 223 nm

and dead time (2.30 min) was determined by a signal of solvent B. Peptide samples were dissolved in water with 0.10% of TFA. The injected sample volume was 20 μm.

QSRR Analysis

To receive molecular structural descriptors of the tested peptides, which were used as independent variables, ACD software (Advanced Chemistry Development, Toronto, Canada) and HyperChem with ChemPlus upgrade software (HyperCube, Waterloo, Canada) were used. The independent variables used in multiple regression equations were selected from a set of 60 molecular structural descriptors. Finally, the following molecular modeling descriptors were employed: refraction coefficient, R_I, bond energy logarithm, log *Bond*, molecular density logarithm, log *Density*, squared energy of LUMO orbital, E^2_{LUMO}.

Experimental TLC data (R_f and R_M values) obtained the following parameters: *Sum* AA_{Rf} – the sum of amino acids' R_f values contained in the individual peptide, log *Sum* AA_{Rf} – logarithm of *Sum* AA_{Rf}, *Sum* AA_{RM} – the sum of amino acids' R_M contained in a defined peptide, log *Sum* AA_{RM} – logarithm of *Sum* AA_{RM}.

The QSRR equation was derived by means of multiple regression analysis with Statistica software (StatSoft Inc., Tulsa, OK, USA) run on a personal computer. Regression coefficients, multiple correlation coefficients, R, standard errors of estimate, s, significance levels of each term, and the whole equation, p, values of the F-test of significance, F, were calculated and used to evaluate the overall accuracy of the statistics of the derived equation.

RESULTS AND DISCUSSION

Several mobile phases were examined in the TLC experiment to find the best conditions for the separation of 20 naturally occurring amino acids (Table 1). Satisfying results in amino acids separation were achieved with mobile phases designated with numbers 1, 2, 5, 6, 7. As the best option for the separation of amino acids, a mobile phase composed of water and ethanol was finally considered. Water and ethanol were mixed in different proportions and the best separation was achieved on NP-TLC plates with the mixture of water and ethanol 95° (20:80 v/v) used as mobile phase (Figure 1). Obtained values of R_f and R_M were next used in QSRR analysis (Table 2).

The best QSRR equation was obtained with the use of experimentally obtained descriptor, *Sum* AA_{RM}. That descriptor was obtained in the TLC experiment with the mobile phase comprised of the mixture of water and ethanol 95° (20:80 v/v). Other independent variables used in QSRR were as

Table 1. Composition of the examined mobile phases

No	Composition	v/v
1	Chloroform:1-buthanol:formic acid	10:31:10
2	Chloroform:methanol:25% water solution of ammonia	20:20:60
3	Water:ethanol:25% water solution of ammonia	10:1:1
4	Water:ethanol 95°:acetic acid	10:10:20
5	Water:ethanol 95°	30:70
6	Water:ethanol 95° with the addition of 1 ml 1-hexyl-3-methylimidazoline tetrafluoroborate for every 100 ml of the mobile phase	30:70
7	Water:2-propanol	30:70
8	Water with 0.1% TFA	100
9	Water with 0.1% TFA:acetonitrile with 0.1% TFA	50:50
10	Water with 0.1% TFA:acetonitrile with 0.1% TFA	20:80
11	1.0 N Hydrochloric acid:ethanol	10:90

following: refraction factor, R_I, logarithm of bond energy, log *Bond*, logarithm of molecular density, log *Density*, squared value of LUMO orbital energy, E^2_{LUMO}. The dependent variable considered in QSRR was retention time, t_R, of 35 peptides analyzed with HPLC (Table 3). The derived QSRR equation has the form:

$$t_R = -58.60(\pm 12.03) - 0.57(\pm 0.09)\ Sum\ AA_{RM} + 8.18(\pm 0.50)\log Bond$$
$$p = 3 \times 10^{-5} \quad p = 1 \times 10^{-6} \quad p = 5 \times 10^{-16}$$
$$- 0.03(\pm 0.01)E^2_{LUMO} - 99.24(\pm 10.42)\log Density + 51.73(\pm 7.97)R_I$$
$$p = 0.0141 \quad p = 2 \times 10^{-10} \quad p = 4 \times 10^{-7}$$
$$R = 0.970; \quad F = 93; \quad s = 1.1181; \quad p < 6 \times 10^{-17} \qquad (1)$$

The description of t_R by the set of applied structural parameters is very good. All the coefficients at the five parameters are statistically significant ($p \leq 0.0141$) as is the whole equation ($p = 6 \times 10^{-17}$). Multiple correlation coefficient, R, standard error of estimate, s, and the value of the F-test of significance, F, all are also very good. Prediction potency of the QSRR model is additionally illustrated by the correlation plot between the calculated and experimental HPLC retention times (Figure 2).

The research confirmed the influence of amino acids present in certain peptides on the retention time, and the possibility of making use of TLC amino acids retention data for the predictions of HPLC retention of peptides based on their chemical structures. The main result of the current study is the proposed QSRR model containing new a descriptor,

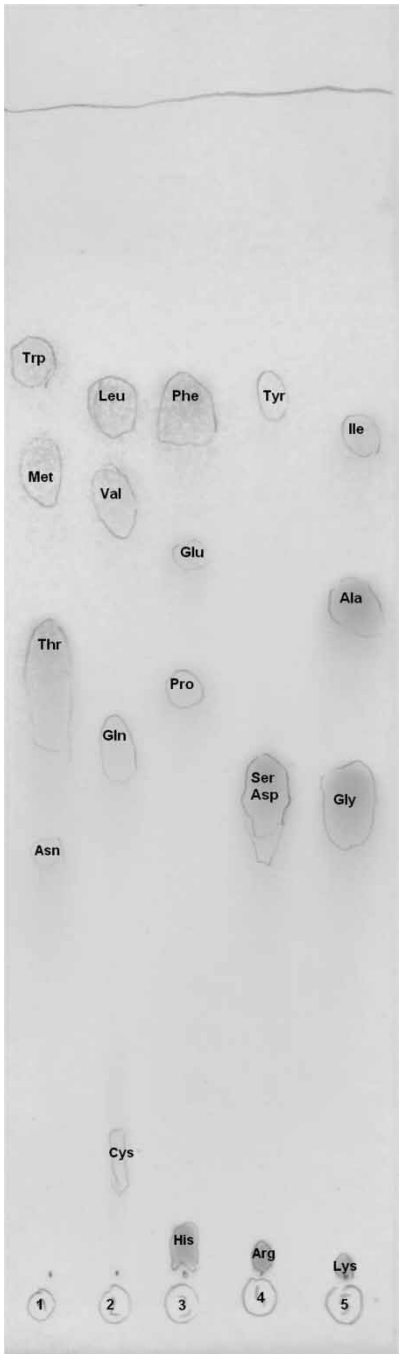

Figure 1. Separation of 20 amino acids on NP-TLC plates with water:ethanol (20:80 v/v) mobile phase.

Table 2. R_f and R_M of amino acids obtained with the use of mobile phases composed of water and ethanol 95° (20:80 *v*/*v*)

No	amino acid	R_f	R_M
1	**A**	0.51	−0.02
2	**R**	0.04	1.39
3	**N**	0.38	0.21
4	**D**	0.36	0.24
5	**C**	0.20	0.61
6	**E**	0.52	−0.03
7	**Q**	0.50	0.00
8	**G**	0.36	0.26
9	**H**	0.02	1.75
10	**I**	0.68	−0.32
11	**L**	0.79	−0.56
12	**K**	0.02	1.74
13	**M**	0.73	−0.43
14	**F**	0.64	−0.26
15	**P**	0.39	0.19
16	**S**	0.32	0.33
17	**T**	0.68	−0.32
18	**W**	0.80	−0.60
19	**Y**	0.62	−0.21
20	**V**	0.85	−0.76

which was obtained from TLC experimental data. The above mentioned TLC system was considered as an optimal one for the separation of amino acids. The proposed mobile phase allows obtaining different R_f values for each amino acid. The diversity of R_f values that was achieved using this TLC system permitted receiving a new structural parameter–the sum of amino acids' R_M values contained in the individual peptide, which can be use as an independent variable to predict retention for any given peptide. The sum of R_M values, corresponding to the individual amino acids in certain peptides, used together with structural parameters obtained in molecular modeling, enabled achieving a new QSRR model, which allows for approximate prediction of gradient reversed-phase HPLC retention time of structurally diversified peptides. The structural parameter, *Sum* AA_{RM}, was obtained in a strictly defined TLC system but it could be used also as an independent variable for every peptide with defined molecular structure in the proposed QSRR equation. Likewise, the above mentioned descriptor, derived QSRR model, was established for a given chromatographic system, but it can be next used for evaluation of retention for every peptide with a known molecular structure and chromatographed in the given HPLC system.

Table 3. Values of descriptors and calculated and experimental values of HPLC retention times, t_R, for peptides used in QSRR study

No	Amino acid sequence	*Sum* AA_{RM}	log *Bond*	E^2_{LUMO}	log Density	R_I	t_R calc (min)	t_R exp (min)	Δt_R (min)
1	AA	−0.0305	−0.5226	65.8878	0.0821	1.492	4.20	2.75	1.45
2	AG	0.2400	−0.6839	66.6533	0.1014	1.497	1.04	2.38	1.34
3	AF	−0.2705	−0.2566	22.2812	0.0871	1.565	11.10	11.87	0.77
4	YL	−0.7735	−0.0545	20.4401	0.0856	1.565	13.24	12.65	0.59
5	GL	−0.3090	−0.2249	68.4362	0.0554	1.488	9.16	10.95	1.79
6	WF	−0.8573	−0.1273	14.0876	0.1202	1.671	14.93	15.60	0.67
7	GHG	2.2587	−0.2841	44.1183	0.1617	1.607	3.55	2.72	0.83
8	LPQIENVKGTEDSGTT-CONH2	0.5581	0.8031	62.5373	0.1329	1.567	13.65	13.00	0.65
9	Ac-NH-CEQDGDPE-CONH2	1.4802	0.7030	62.7557	0.1711	1.588	9.59	10.48	0.89
10	LPPGPAVVDLTEKLEGQGG-CONH2	−0.0298	0.8960	63.2230	0.1153	1.559	16.06	16.45	0.39
11	DRVYIHPF	2.0234	0.6363	20.3391	0.1553	1.663	15.45	15.15	0.30
12	KETS	1.7178	0.1029	65.2298	0.1383	1.562	6.38	4.20	2.18
13	VAKETS	0.9456	0.3015	64.7858	0.1186	1.550	9.79	8.70	1.09
14	HTVAKETS	2.3763	0.4605	44.1512	0.1339	1.575	10.68	9.50	1.18
15	MAGAAAAG-NH2	0.0084	0.2990	64.5396	0.1031	1.542	11.44	10.10	1.34
16	SKPKTNMKHMAGAAAAG-NH2	6.9584	0.7817	38.2209	0.1193	1.576	12.37	11.38	0.99
17	Ac-HNPGYPHNPGYPHNPGYPHNPGYP-NH2	9.5263	1.0230	20.4750	0.1664	1.649	12.51	13.23	0.72
18	TLSYPLVSVVSESLTPER-NH2	−1.7992	0.8832	20.5365	0.1644	1.644	17.77	17.72	0.05
19	EVHHQKLVFFAEDVGSNK-NH2	4.5875	0.8787	22.1658	0.1219	1.582	15.05	14.63	0.42
20	EVHHQKLVFFAQDVGSNK-NH2	4.6186	0.9018	46.6832	0.1199	1.582	14.68	14.45	0.23
21	DAEFGHDSG-NH2	2.7691	0.4915	0.0001	0.1661	1.607	10.48	10.93	0.45
22	EVRHQKLVFF-NH2	2.2580	0.7142	0.0076	0.1430	1.643	16.75	15.53	1.22

(*continued*)

Table 3. Continued

No	Amino acid sequence	*Sum* AA_{RM}	log *Bond*	E^2_{LUMO}	log Density	R_I	t_R calc (min)	t_R exp (min)	Δt_R (min)
23	LVFF-NH2	−1.8318	0.2778	22.1787	0.0607	1.561	18.78	17.15	1.63
24	GSNKGAIIGLM-NH2	1.3868	0.6452	63.0656	0.0927	1.544	14.66	15.47	0.81
25	KEGVLY-NH2	0.4341	0.4153	61.5414	0.1202	1.577	12.35	12.78	0.43
26	EGVLY-NH2	−1.3063	0.3132	20.5207	0.1326	1.584	12.87	13.22	0.35
27	GVLY-NH2	−1.2752	0.2432	20.5231	0.1166	1.582	13.76	13.08	0.68
28	Ac-ETHLHWHTVAK-NH2	4.3805	0.7160	27.8080	0.1290	1.606	14.20	13.78	0.42
29	HT	1.4208	−0.3184	43.8094	0.1550	1.603	4.21	2.48	1.73
30	WHT	0.8287	−0.0133	37.7250	0.1569	1.675	1.77	11.62	0.85
31	HLHWHT	3.7608	0.4416	27.8098	0.1480	1.653	12.86	13.10	0.24
32	ETHLHWHT	3.4124	0.5419	27.8096	0.1538	1.640	12.62	12.87	0.25
33	EVRHQK	4.0897	0.4224	38.3124	0.1790	1.660	9.48	8.82	0.66
34	Ac-EVHHQKLVFF	2.6170	0.6870	22.1732	0.1086	1.581	15.87	16.42	0.55
35	Ac-DAEFGH	1.9450	0.3539	22.0973	0.1511	1.594	9.99	12.25	2.26

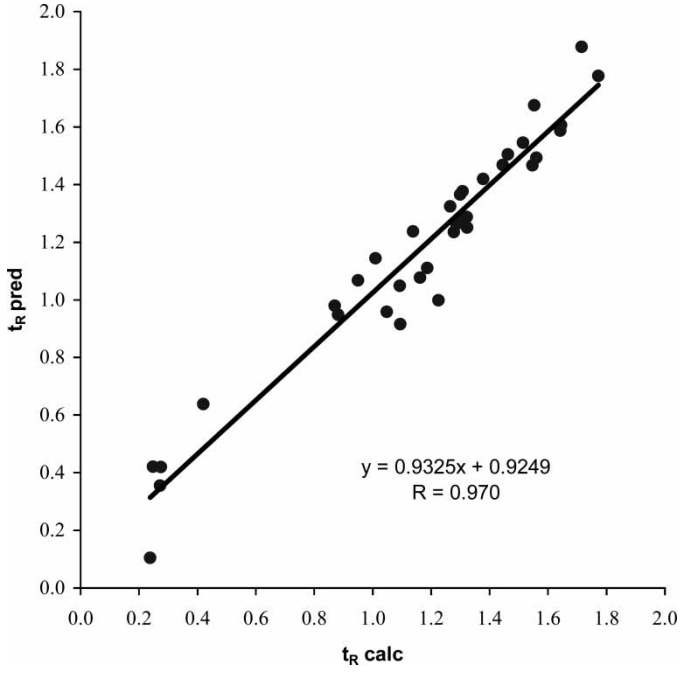

Figure 2. Correlation between the t_R calculated by QSRR (Eq. 1) and experimental t_R for a set of 35 peptides studied.

The obtained QSRR equation can be considered as an alternative tool used for predictions of HPLC retention of peptides during a protein identification process in proteomics, improving the confidence of peptide identification and increasing the number of the correct identifications.[14,15,18]

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REFERENCES

1. Hunter, T.C.; Andon, N.L.; Koller, A.; Yates, J.R., III; Haynes, P.A. The functional proteomics toolbox: methods and applications. J. Chromatogr. B **2002**, *782*, 165–181.
2. Sherma, J.; Fried, B. *Handbook of Thin–Layer Chromatography*; Marcel Dekker, Inc: New York, 1996.
3. Casal, V.; Martin-Alvarez, P.J.; Herraiz, T. Comparative prediction of retention behavior of small peptides in several reversed-phase high-performance liquid

chromatography columns by using partial least squares and multiple linear regression. Anal. Chim. Acta. **1996**, *626*, 77–84.
4. Meek, J.L. Prediction of peptide retention times in high-pressure liquid chromatography on the basis of amino acid composition. Proc. Natl. Acad. Sci. **1980**, *77*, 1632–1636.
5. Browne, C.A.; Bennett, H.P.J.; Salomon, S. The isolation of peptides by high-performance liquid chromatography using predicted elution positions. Anal. Biochem. **1982**, *124*, 201–208.
6. Guo, D.; Mant, C.T.; Taneja, A.K.; Parker, J.M.R.; Hodges, R.S. Prediction of peptide retention times in reversed-phase high-performance liquid chromatography. J. Chromatogr. **1986**, *359*, 499–532.
7. Mant, C.T.; Zhou, N.E.; Hodges, R.S. Correlation of protein retention times in reversed-phase chromatography with polypeptide chain length and hydrophobicity. J. Chromatogr. **1989**, *476*, 363–37.
8. Wang, Y.; Gu, X.; Zhang, J.; Zhang, X. Predict of peptide retention in RP-LC. Chromatographia **2005**, *62*, 385–392.
9. Kaliszan, R. *Structure and Retention in Chromatography: A Chemometric Approach*; Harwood Academic Publishers: Amsterdam, 1997.
10. Kaliszan, R.; Bączek, T.; Buciński, A.; Buszewski, B.; Sztupecka, M. Prediction of gradient retention from the linear solvent strength (LSS) model, quantitative structure-retention relationship (QSRR), and artificial neural networks (ANN). J. Sep. Sci. **2003**, *26*, 271–282.
11. Kaliszan, R. Quantitative structure-retention relationships. Anal. Chem. **1992**, *64*, 619A–631A.
12. Bączek, 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.
13. Bączek, T. Chemometric evaluation of relationships between retention and physicochemical parameters in term of multidimensional liquid chromatography of peptides. J. Sep. Sci. **2006**, *29*, 547–554.
14. Kaliszan, R.; Bączek, T.; Cimochowska, A.; Juszczyk, P.; Wiśniewska, K.; Grzonka, Z. Prediction of high-performance liquid chromatography retention of peptides with the use of quantitative structure-retention relationships. Proteomics **2005**, *I*, 409–415.
15. Bączek, T.; Wiczling, P.; Marszałł, M.P.; Vander Heyden, Y.; Kaliszan, R. Prediction of peptides retention at different HPLC conditions from multiple linear regression models. J. Proteome Res. **2005**, *4*, 555–563.
16. Kaliszan, R. *Quantitative Structure-Retention Relationship*; Wiley: New York, 1987.
17. Bączek, T.; Sparzak, B. Ionic liquids as novel solvent additives to separate peptides. Z. Naturforsch **2006**, *61c*, 827–832.
18. Put, R.; Daszykowski, M.; Bączek, T.; Vander Heyden, Y. Retention prediction of peptides based on uninformative variable elimination by partial least squares. J. Proteome Res. **2006**, *5*, 1618–1625.

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