Comparison of Different Retention Models in Normal- and Reversed-Phase Liquid Chromatography with Binary Mobile Phases

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Abstract

The dependence on mobile phase composition of the retention of selected test analytes in different normal- and reversed-phase chromatographic systems is studied. The aim of this study is to compare the performance of six valuable retention models reported in the literature with a new empirical equation, first introduced in this study. All of these models are compared for different thin-layer chromatographic and high-performance liquid chromatographic systems by use of three criteria: the sum of the squared differences between the experimental and theoretical data, approximation of the standard deviation, and the Fisher test.

Introduction

Binary mobile phases consisting of mixtures of weak and strong solvents are widely used in thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Mobile phase composition determines the retention volume and retention time of solutes in both normal-phase (NP) and reversed-phase (RP) chromatography. Alteration of the composition and the nature of mobile phases enables tuning of the retention for the separated analytes and optimization of the chromatographic process. For example, in RP-liquid chromatography an important constituent of the mixed mobile phase is the highly polar solvent (e.g., water). A less polar solvent (e.g., methanol and acetonitrile) is used as an organic modifier, which is added to control the process of solute elution.

Typically, prediction of the retention time is based on the expected dependence of the retention factor (k) on mobile phase composition. In column chromatography, k is defined as:

$$k = \frac{t_r - t_o}{t_o} \qquad \text{Eq. 1}$$

where t_r and t_0 are the retention times of the analyte and an unretained solute, respectively.

In planar chromatography, the retardation factor (R_f) is most frequently used to measure retention and is related to k by the equation:

$$R_f = \frac{1}{1 + k} \qquad \text{Eq. 2}$$

Several different retention models have been reported in the literature (1–6,10). The purpose of this study was to compare the performance of seven retention models for the prediction of retention in different planar and column chromatographic systems.

Snyder derived the linear relationship (1,2) as:

$$\ln k = p_1 - p_2 \cdot \mathrm{j} \qquad \text{Eq. 3}$$

where j is the mole fraction of the organic modifier in the binary mobile phase and p_1 and p_2 are constants. Similarly, Snyder and Soczewi?ski (3) proposed the equation:

$$\ln k = p_1 - p_2 \cdot \ln \mathrm{j} \qquad \text{Eq. 4}$$

and Hsieh and Dorsey (4) suggested the following form of the retention model:

$$\ln k = p_1 \cdot \ln\left(\frac{1}{\mathrm{j}}\right) + p_2 \qquad \text{Eq. 5}$$

Mathematically, however, equation 5 is identical to equation 4; therefore, Hsieh and Dorsey's model was not included in this study.

The Langmuir-type relationship between k and the amount of organic modifier in a binary mobile phase was first proposed by Row et al. (1), who assumed that adsorption of a modifier can be described by the use of the Langmuir isotherm. The final form of this expression is:

$$k = p_1 + p_2 \cdot \frac{1}{\mathrm{j}} \qquad \text{Eq. 6}$$

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One of the most accurate (and therefore most widely used) equations to predict solute retention was proposed by Schoenmakers (5):

$$\ln k = p_1 \cdot \varphi^2 + p_2 \cdot \varphi + p_3 \qquad \text{Eq. 7}$$

Two relationships between the R_f and mobile-phase composition for NP- and RP-planar chromatography were proposed by Kowalska (6). For NP chromatography it was:

$$Rf = p_1 \cdot \sqrt{\varphi} + p_2 \cdot (1 - \varphi) + p_3 \qquad \text{Eq. 8}$$

and for RP chromatography it was:

$$Rf = p_1 \cdot \sqrt{\varphi} + p_2 \cdot \sqrt{(1 - \varphi)} + p_3 \qquad \text{Eq. 9}$$

where p_1, p_2, and p_3 are constants.

The adsorption–partition model for the description of the retention coefficient as a function of the mixed mobile phase composition was stated by Kaczmarski et al. (5,10). For RP chromatography, k can be expressed as:

$$k = exp(p_1 + p_2 \cdot \varphi) + \frac{1}{p_3 \cdot \varphi + p_4 \cdot (1 - \varphi)} \qquad \text{Eq. 10}$$

where p_1, p_2, p_3, and p_4 are constants. For NP chromatography the relationship proposed by Kaczmarski et al. is:

$$k = \frac{1}{p_1 \cdot \varphi + p_2 \cdot (1 - \varphi)} \qquad \text{Eq. 11}$$

In this study, a novel empirical relationship for the accurate prediction of analyte retention in planar and column chromatography is:

$$\ln k = p_1 - p_2 \cdot \varphi^{p3} \qquad \text{Eq. 12}$$

It is recognized that the Schoenmakers relationship (originally devised for HPLC) can be successfully applied to TLC, also (7). This is why we decided to evaluate the performance of all of these models for the prediction of retention in both TLC and HPLC. These models were compared for different TLC and HPLC systems by using the sum of the squared differences between experimental and theoretical retention data:

$$SUM = \sum_i \left(k_{exp}(i) - k_{theor}(i) \right)^2 \qquad \text{Eq. 13}$$

approximation of the standard deviation (SD):

$$SD = \sqrt{\frac{SUM}{LD - L}} \qquad \text{Eq. 14}$$

Table I. Test Analytes, Mobile Phases, Ranges of Modifier Mole Fractions, and the TLC Mode Used

No.	Test analyte	Mobile phase composition	Range of modifier mole fraction	TLC mode
1	ortho-Chloronitrobenzene	n-Hexane–acetone	0.03–0.64 (acetone)	NP-TLC
2	meta-Chloronitrobenzene	n-Hexane–acetone	0.03–0.64 (acetone)	NP-TLC
3	para-Chloronitrobenzene	n-Hexane–acetone	0.03–0.64 (acetone)	NP-TLC
4	ortho-Nitrotoluene	CCl4–chloroform	0.04–0.28 (chloroform)	NP-TLC
5	meta-Nitrotoluene	CCl4–chloroform	0.04–0.28 (chloroform)	NP-TLC
6	para-Nitrotoluene	CCl4–chloroform	0.04–0.28 (chloroform)	NP-TLC
7	Sulph. deriv., quercetin	Water–methanol	0.04–0.82 (methanol)	RP-TLC
8	Quercetin	Water–methanol	0.15–0.82 (methanol)	RP-TLC
9	1-Naphthylamine*	Water–methanol	0.50–1.00 (v/v, methanol)	RP-TLC
10	8-Methylquinoline*	Water–methanol	0.50–1.00 (v/v, methanol)	RP-TLC

* The experimental data were reported in the literature (10) as volume proportions rather than mole fractions.

Table II. Test Analytes, Mobile Phases, Ranges of the Modifier Mole Fractions, and the HPLC Columns Used

No.	Test analyte	Mobile phase composition	Range of modifier mole fraction	Column
1	Chrisin	Water–methanol	0.30–1.00 (methanol)	250- × 4.6-mm, 5-µm Hypersil BDS C18
2	Quercetin	Water–methanol	0.30–1.00 (methanol)	250- × 4.6-mm, 5-µm Hypersil BDS C18
3	Apigenin*	Water–methanol†	0.31–0.64 (methanol)	250- × 4.5-mm, 7-µm LiChrosorb C-8 (Merck)
4	Chryseriol*	Water–methanol†	0.31–0.64 (methanol)	300- × 3.9-mm, 10-µm Phenyl µBondapak (Waters)
5	Apiin*	Water–methanol†	0.05–0.40 (methanol)	300- × 3.9-mm, 10-µm Cyano µBondapak (Waters)
6	Flavonol*	Water–methanol†	0.31–0.64 (methanol)	300- × 3.9-mm, 10-µm µBondapak C-18 (Waters)
7	Ethylbenzene‡	*n*-Hexane–THF	8×10^{-5}–3.21×10^{-3} (THF)	120- × 2-mm, 10-µm Silasorb-NH_2, Milichrom
8	Hexylbenzene‡	*n*-Hexane–THF	8×10^{-5}–3.21×10^{-3} (THF)	120- × 2-mm, 10-µm Silasorb-NH_2, Milichrom
9	*o*-Xylene‡	*n*-Hexane–THF	8×10^{-5}–3.21×10^{-3} (THF)	120- × 2-mm, 10-µm Silasorb-NH_2, Milichrom
10	1,2,4,5-Tetramethylbenzene‡	*n*-Hexane–THF	8×10^{-5}–3.21×10^{-3} (THF)	120- × 2-mm, 10-µm Silasorb-NH_2, Milichrom
11	1-Naphthol§	*2*-Propanol–*n*-hexane	0.5–1.0 (propanol) (v/v)	119- × 4-mm, 5-µm LiChrospher 100 CN (Merck)
12	*m*-Cresol§	*2*-Propanol–*n*-hexane	0.5–1.0 (propanol) (v/v)	119- × 4-mm, 5-µm LiChrospher 100 CN (Merck)
13	Dibenzo-24-crown-8§	Water–methanol	0.6–1.0 (methanol) (v/v)	119- × 4-mm, 5-µm LiChrospher 100 RP-8 (Merck)
14	Dibenzo-24-crown-8§	Water–methanol	0.6–1.0 (methanol) (v/v)	119- × 4-mm, 5-µm LiChrospher 100 RP-8e (Merck)
15	Dibenzo-24-crown-8§	Water–methanol	0.6–1.0 (methanol) (v/v)	119- × 4-mm, 5-µm LiChrospher 100 RP-18 (Merck)
16	Dibenzo-24-crown-8§	Water–methanol	0.6–1.0 (methanol) (v/v)	119- × 4-mm, 5-µm LiChrospher 100 RP-18e (Merck)

* Experimental data from reference 9.
† Acetic acid as the acidic modifier.
‡ Experimental data from reference 3.
§ Experimental data from reference 5, in which volume proportions rather than mole fractions are given.

and the Fisher test:

$$F = \frac{\sum_i \frac{\left(k_{exp}(i) - \sum_i \frac{k_{exp}(i)}{LD}\right)^2}{LD-1}}{\sum_i \frac{(k_{exp}(i) - k_{theor}(i))^2}{LD-L}} \qquad \text{Eq. 15}$$

where LD is the number of experimental points, L the number of estimated parameters, and $i = 1 \ldots LD$.

Model variables (p_i) were estimated by the minimization of the sum of the squared differences between experimental and theoretical data by use of the Marquardt method (8).

Models 3–10 were compared by using our own experimental results and retention data also taken from the literature [and contained in studies by Kahie et al. (9), Lanin et al. (3), and Kaczmarski et al. (5,10)].

Experimental

Chrysin and quercetin were purchased from Sigma (St. Louis, MO). LiChrosolv chromatographic-grade methanol and water, other chromatographic-grade solvents, and chloronitrobenzene

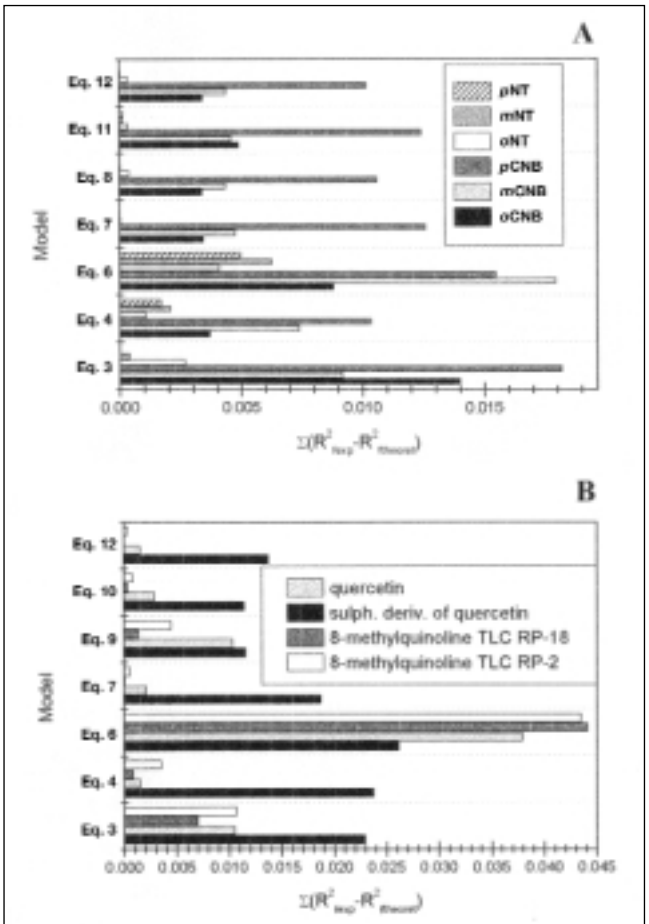

Figure 1. Graphical comparison of the sums of the squared differences between experimental and theoretical retention data: (A) NP-TLC systems and (B) RP-TLC systems.

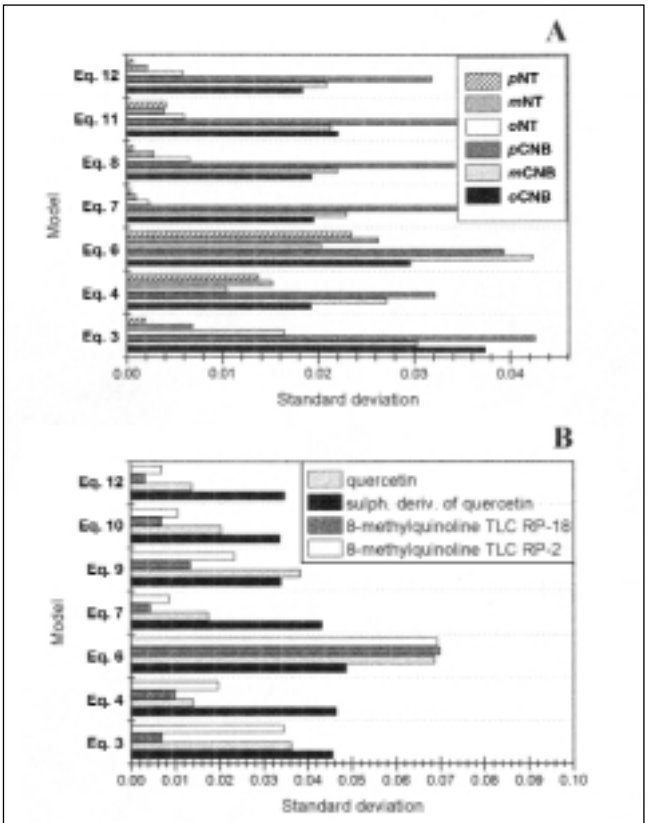

Figure 2. Graphical comparison of the numerical values of the SD: (A) NP-TLC systems and (B) RP-TLC systems.

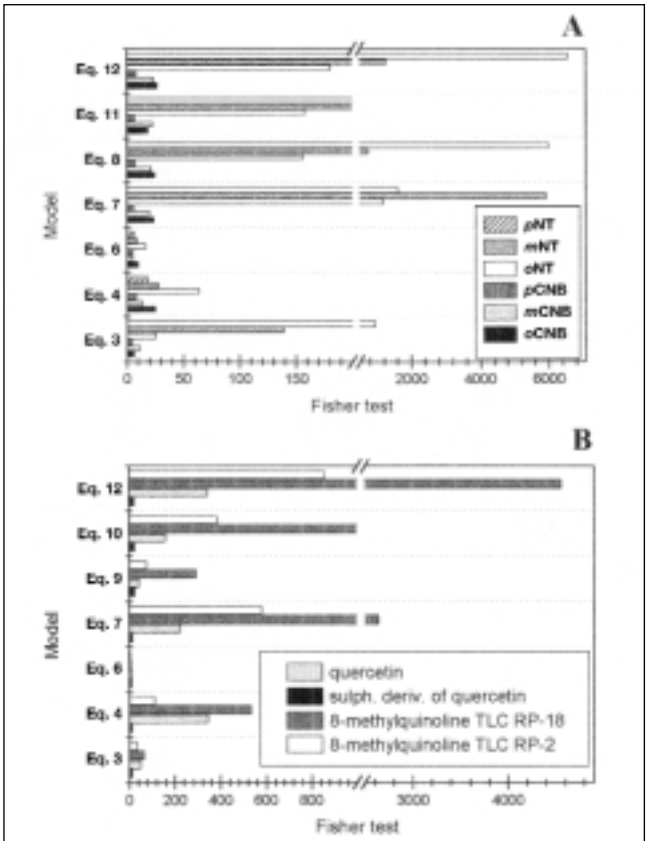

Figure 3. Graphical comparison of the numerical values of the Fisher test: (A) NP-TLC systems and (B) RP-TLC systems.

and nitrotoluene isomers were purchased from E. Merck (Darmstadt, Germany). The sodium salt of quercetin sulfonic acid (11) was a gift from the Department of Inorganic and Analytical Chemistry, Rzeszów University of Technology.

In order to verify the performance of the retention models, our TLC and HPLC experiments were performed with the following chromatographic systems and equipment.

TLC

Table I specifies the samples, mobile phases, range of modifier mole (or volume) fractions (separately for each mixed mobile phase), and the mode of TLC employed (NP- or RP-TLC).

TLC was performed on either RP-18 (cat. #105559) or silica gel 60 F_{254} (cat. #105554) aluminum-backed chromatographic plates from Merck. In our experiments all of the TLC plates were developed to a distance of 10 cm.

HPLC

Table II specifies the samples, mobile phases, range of the modifier mole (or volume) fractions (separately for each mobile phase), and the mode of HPLC employed (NP- or RP-HPLC columns).

HPLC was performed with a Merck–Hitachi Model L-7100 La Chrom pump, a Merck–Hitachi Model L-7455 DAD La Chrom detector, a Merck–Hitachi Model D-7000 La Chrom interface, a Merck–Hitachi Model L-7350 column oven, a Merck Model L-7612 solvent degasser, a 20-mL injection loop, and a Hypersil BDS C_{18} chromatography column (250- ¥ 4.6-mm, average particle diameter of 5 mm). The mobile phase flow rate was 1 mL/min, the absorbance was measured at 250 nm, and the column temperature was 20 C. Elution was performed in the isocratic mode.

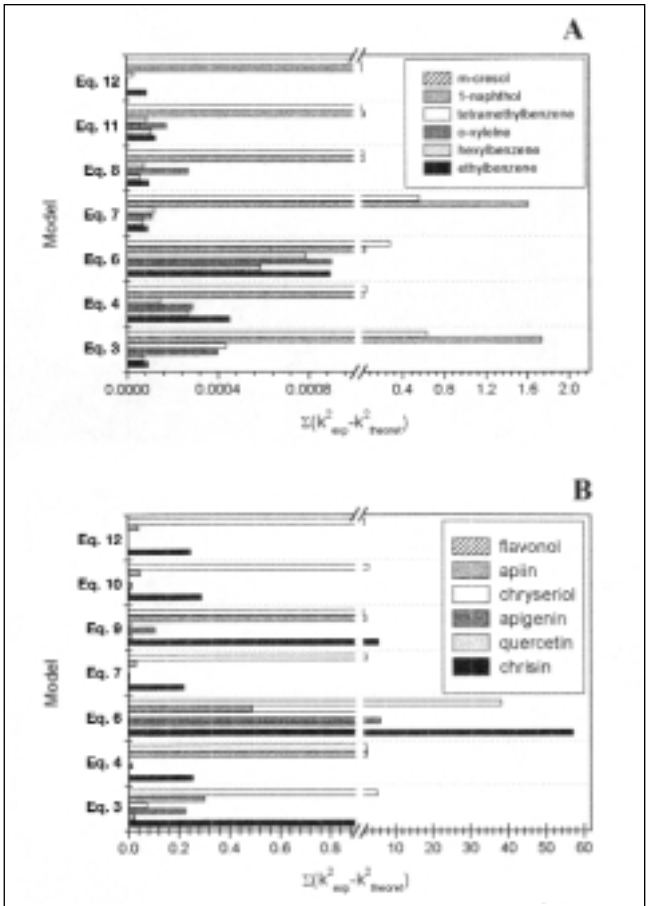

Figure 4. Graphical comparison of the sums of the squared differences between experimental and theoretical retention data: (A) NP-HPLC systems and (B) RP-HPLC systems.

Results and Discussion

TLC

The results from our investigations of different TLC systems are presented in Figures 1–3. These show, respectively, the sums of the squared differences between the experimental and theoretical data, the SDs, and the Fisher test values obtained when the different retention models were compared.

The results obtained for the different TLC systems demonstrate that the best agreement between the experimental and calculated R_f was obtained by using equation 12. This model provides an accurate description of the R_f coefficient for most of the test solutes and chromatographic systems studied. Slightly less accurate results were obtained by the use of equations 4, 7, 8, and 11 for NP-TLC systems and equations 4, 7, 9, and 10 for RP-TLC systems. The other equations performed much less accurately.

HPLC

The results from our investigations of different HPLC systems are presented in Figures 4–9. In a fully analogous form with that

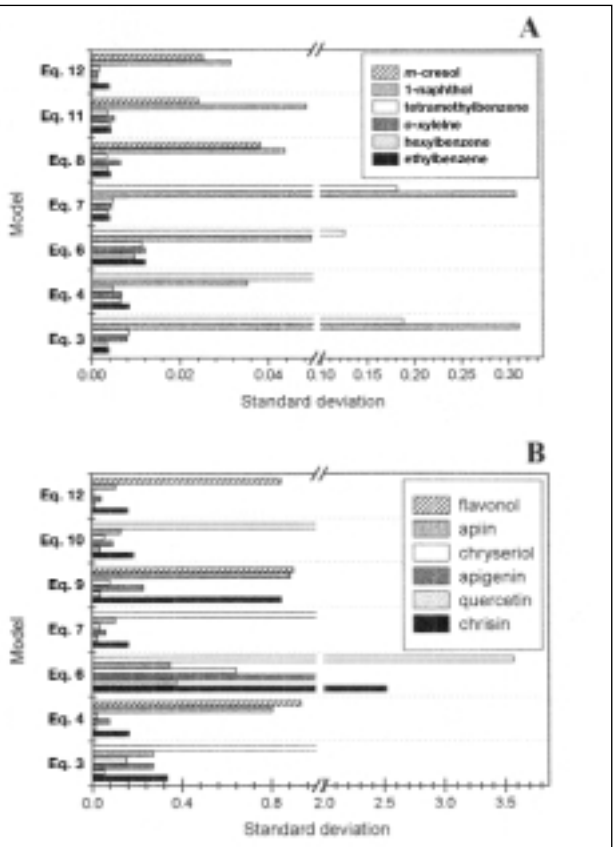

Figure 5. Graphical comparison of the numerical values of the SD: (A) NP-HPLC systems and (B) RP–HPLC systems.

given in Figures 1–3, the sums of the squared differences between the experimental and theoretical data, the SDs, and the Fisher test values obtained for the different retention models were also compared.

From the results obtained from the comparison of the seven retention models (equations 3–12), it can be concluded that the three-parameter model proposed in this study (equation 12) provided excellent agreement between the experimental and theoretical retention data for most of the NP and RP chromatographic systems studied. This empirical model was established on the basis of many different experimental data (not given in this study). It performed equally well both at high and low levels of the organic modifiers. For the RP-HPLC of crown compounds as test analytes (Figures 7–9), the best simulations were obtained by the use of equation 10 (5,10) and equation 7 (the Schoenmakers equation). The outstanding results obtained by the use of equation 10 seemed to convincingly point to the dual effect (adsorption and partition) of the retention process.

The other cited retention models performed considerably poorer, thus leading to much higher computational errors. The worst results for NP- and RP-HPLC were obtained by the use of equations 3 and 6. For typical NP- and RP-HPLC systems, equations 4, 7, 8, 9, and 10 provided quite similar results to one another.

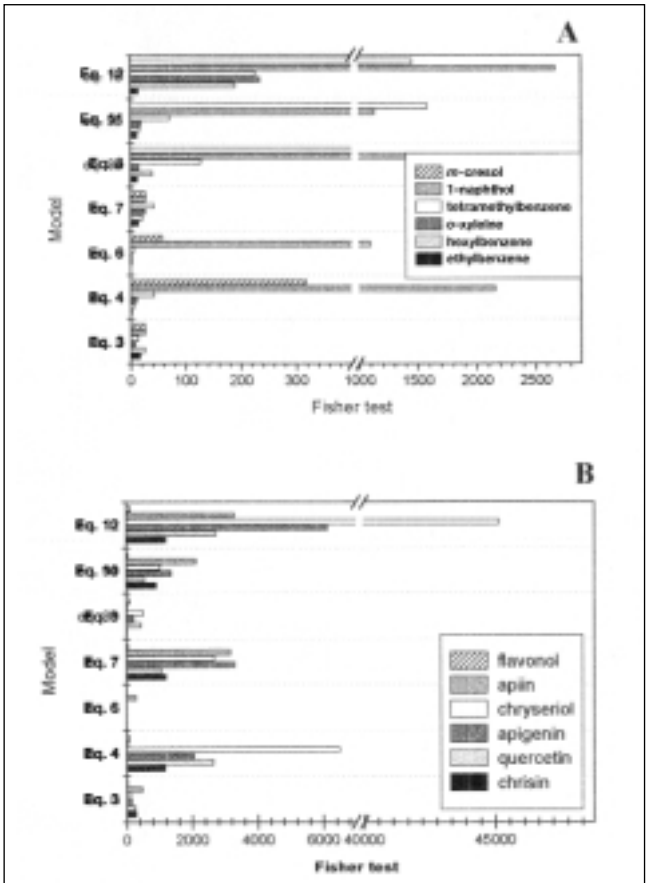

Figure 6. Graphical comparison of the numerical values of the Fisher test: (A) NP-HPLC systems and (B) RP-HPLC systems.

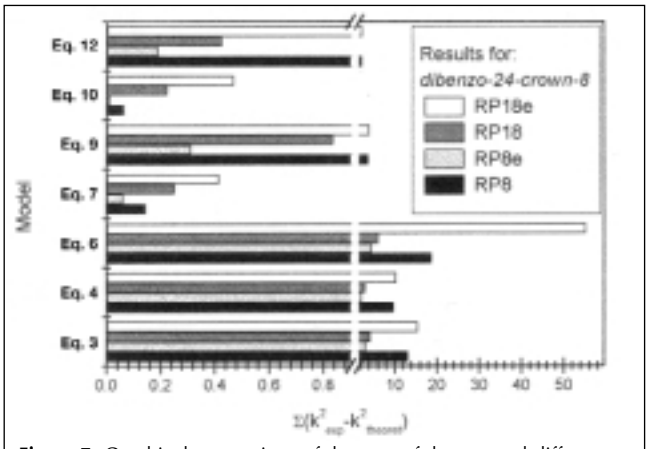

Figure 7. Graphical comparison of the sum of the squared differences between the experimental and theoretical retention data for dibenzo-24-crown-8 (as an example of a test solute) chromatographed in different RP-HPLC systems.

Conclusion

In this study seven retention models were examined by fitting them to retention data obtained for a set of different test solutes

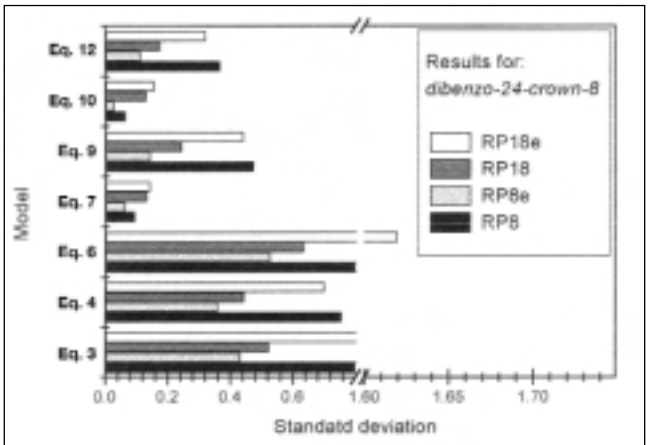

Figure 8. Graphical comparison of the numerical values of the SD for dibenzo-24-crown-8 (as an example of a test solute) chromatographed in different RP-HPLC systems.

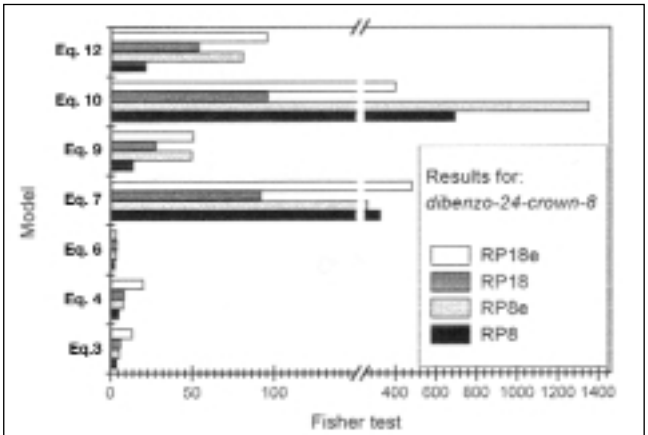

Figure 9. Graphical comparison of the Fisher test for dibenzo-24-crown-8 (as an example of a test solute) chromatographed in different RP-HPLC systems.

by the use of a wide variety of TLC and HPLC systems. For most of the chromatographic systems considered, the newly proposed equation 12 proved a more reliable approach than other retention models published in the literature. The usefulness of equation 12 will be further tested in the future by the mathematical modeling of chromatographic columns.

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