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Modelling of Retention Factors of Analytes in Chromatography with Ternary Solvent Mobile Phases

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A Jouyban–Acree model has been applied to search for mathematical representation of retention factors of phenobarbital, phenytoin, and carbamazepine in mobile phases containing water and organic modifiers: methanol, acetonitrile, acetone, and tetrahydrofuran. An average percentage deviation (APD) of experimental and calculated data has been adopted as the criterion of accuracy of the proposed model. It has been proved that the Jouyban--Acree model provides accurate results and can be applied in practice to speed up analytical procedure when ternary solvent mobile phases are used.

Model Joubana–Acree zastosowano do matematycznego wyznaczania współczynników retencji fenobarbitalu, fenytoiny i karbamazepiny przy ich chromatografowaniu z użyciem faz ruchomych zawierających wodę i modyfikatory organiczne: metanol, acetonitryl, aceton i tetrahydrofuran. Jako kryterium dokładności zaproponowanego modelu obliczeń zastosowano średnie odchylenie procentowe (APD) danych doświadczalnych i obliczonych. Wykazano, że model Joubana-Acree pozwala uzyskiwać dokładne wyniki i może być stosowany w praktyce do przyspieszania analiz przy użyciu trójskładnikowych faz ruchomych.

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Liquid chromatography, especially reversed-phase HPLC, has rapidly developed and become popular as a reliable and versatile analytical tool for the separation and quantification of analytes in chemical analysis. Many validated chromatographic methods employ ternary solvent mobile phases [1–10]. For instance, Johansen *et al.* used acetonitrile-tetrahydrofuran-phosphate buffer mixtures (22:6.5:71.5) and ODS column to analyse carbamazepine, phenytoin, and phenobarbital in plasma [6]. If a decimal figure appears in the solvent composition, the number of required experiments will be increased. In chromatographic separations, optimisation of the mobile phase composition is traditionally carried out applying a trial-and-error method, or assuming that a logarithm of retention factor is a weighted average of the solute's logarithms of retention factors in each of the solvents composing a mobile phase.

The number of possible combinations of solvents in ternary mixtures is very large and the trial-and-error approach is time-consuming and also expensive. Retention factors calculated applying simple arithmetic/geometric weighted averages of mobile phase composition are superior to the trial-and-error method. However, for the systems of pharmaceutical importance, the calculated value may still differ significantly from the observed behaviour of the solute. The aim of this communication was to report retention factors (k) of the studied analytes and to propose mathematical model for calculating them with respect to the composition of the mobile phase. Applicability of this model has been proved using k values of three anti-epileptic drugs in two sets of aqueous ternary solvent mobile phases.

EXPERIMENTAL

Methanol, acetonitrile, acetone, tetrahydrofuran, potassium hydrogen phosphate, and sodium nitrite were purchased from Merck (Darmstadt, Germany). Drugs were provided by Sobhan (Rasht, Iran), Daroupakhsh (Teheran, Iran) and Ruzdarou (Teheran, Iran) pharmaceutical companies. The liquid chromatographic system comprised a Maxi–Star K–1000 pump, a 4-channel K–5004 degasser, a Well–Chrom K–2500 UV detector, and a Well–Chrom interface box (Knauer Co. Germany). A Nova–Pak C_{18} reversed--phase column (4.6 × 250 mm) was used (Waters company, Massachusett, USA). An ultrasonic water bath served as a degasser (Liarre Co., Bologna, Italy). Millipore pump and GVHP filters (0.22 μm, Millipore, Ireland) were used for mobile phase filtration. 4 mmol L^{-1} phosphate buffer (pH = 6) was prepared by dissolving an appropriate amount of potassium hydrogen phosphate in doubly distilled water; pH was adjusted with orthophosphoric acid. Mobile phases were prepared by mixing appropriate volumes of buffer solution and organic solvents. After mixing, the obtained mobile phase was filtered and degassed for 15 min in an ultrasonic bath. After that, it was passed through the column at 1 mL min^{-1} flow rate to condition the system (30 min). After conditioning, 20 μL of the analytes (100 ppm) were injected *via* an injection loop. k values were evaluated from the retention times of the analytes, t_R, according to the formula: $k = \frac{(t_R - t_0)}{t_0}$, where t_0 is the retention time of the unretained compound. A 50 ppm sodium nitrite solution was used to measure t_0. All measurements were repeated at least three times. UV detection was performed at the wavelenght of 220 nm.

RESULTS AND DISCUSSION

A Jouyban–Acree model [11] has been successively applied to quantify such physicochemical properties of the solute as solubility [12], acid dissociation constants [13], electrophoretic mobility [14], and others [15] that are usually influenced by the solvent composition. General representation of physicochemical properties of the solute dissolved in ternary solvent mixtures is:

$$\ln PCP_m = f_1 \ln PCP_1 + f_2 \ln PCP_2 + f_3 \ln PCP_3 + f_1 f_2 \sum_{i=0}^{q} K_i (f_1 - f_2)^i + f_1 f_3 \sum_{i=0}^{q} K_i' (f_1 - f_3)^i + f_2 f_3 \sum_{i=0}^{q} K_i^* (f_2 - f_3)^i + f_1 f_2 f_3 \sum_{i=0}^{q} K_i''' (f_1 - f_2 - f_3)^i \quad (1)$$

where PCP_m, PCP_1, PCP_2 and, PCP_3 are numerical values of physicochemical properties of the mixture and solvents 1–3, respectively, f denotes volume (mass or mole) fractions of solvents 1–3 in the mixture, and K_i terms represent the model constants. The adopted model for calculating k values of the analytes is as follows:

$$\ln k_m = f_1 \ln k_1 + f_2 \ln k_2 + f_3 \ln k_3 + f_1 f_2 \sum_{i=0}^{q} L_i (f_1 - f_2)^i + f_1 f_3 \sum_{i=0}^{q} L_i' (f_1 - f_3)^i + f_2 f_3 \sum_{i=0}^{q} L_i^* (f_2 - f_3)^i + f_1 f_2 f_3 \sum_{i=0}^{q} L_i''' (f_1 - f_2 - f_3)^i \quad (2)$$

where subscripts m, 1, 2 and 3 refer to the mixed solvent and solvents 1–3 composing the mobile phase, L_i terms are the model constants. In some cases, k values in monosolvent mobile phases, *i.e.* k_1, k_2 and k_3, can not be determined numerically. Then, Equation (2) shall be appropriately rearranged:

$$\ln k_m = J_1 f_1 + J_2 f_2 + J_3 f_3 + f_1 f_2 \sum_{i=0}^{q} L_i (f_1 - f_2)^i + f_1 f_3 \sum_{i=0}^{q} L_i' (f_1 - f_3)^i + f_2 f_3 \sum_{i=0}^{q} L_i^* (f_2 - f_3)^i + f_1 f_2 f_3 \sum_{i=0}^{q} L_i''' (f_1 - f_2 - f_3)^i \quad (3)$$

where J_1–J_3 are the model constants. J_1, J_2, J_3 and L_i terms could be calculated by regressing k_m against f_1, f_2, f_3, f_1f_2, $f_1f_2(f_1–f_2)$, $f_1f_2(f_1–f_2)_2$, *etc.* Equations (2) and (3) were derived on the basis of the fact that retention factor is a partition coefficient, the numerical value of which is determined by the difference in chemical potentials of the solute in the mobile and stationary phases. The corresponding formula describing

chemical potential of the solute dissolved in mixed solvents have been derived previously [16] applying a two-body and three-body interactive mixing model. In the present case, it is required to present both formulas as mathematical representations rather than equations derived from a rigorous thermodynamic model. This is particularly true for all mathematical representations that employ more than a few model constants. Model constants lose their physical meaning as their number introduced into the mathematical expression increases.

Calculated k values were compared with the experimental (observed) values and a mean of the absolute percentage deviation (APD) served as an accuracy criterion. The APD was calculated as follows:

$$APD = \frac{100}{N} \sum \frac{|Calculated - Observed|}{Observed} \tag{4}$$

where N is the number of experimental data points in each set.

k values of the analytes studied in different buffer and organic modifier volume fractions are listed in Table 1. Our aim was to measure k numbers of the analytes in mono-solvent mobile phases. However, very broad peaks were obtained using pure organic modifiers or aqueous buffer solution as mobile phases. Generally, the higher the water (buffer) content in the mobile phase, the longer retention (the higher retention factor) of the analytes was observed. In most cases studied in this work, phenobarbital was eluted first, and was followed by phenytoin and carbamazepine, successively.

Log k values corresponding to particular analytes were applied to Equation (3) with q = 1. The back-calculated k values were subsequently used to compute APDs. The details of the data sets, the number of data points in each set, APDs for the proposed model, correlation coefficients (R), and F values are presented in Table 2. Minimum and maximum APDs are 5.0% (phenytoin in water + tetrahydrofuran + + acetonitrile) and 8.4% (carbamazepine in water + tetrahydrofuran + acetone). Average APD equals 7.0 ± 1.3%. Higher R and F values confirm that the applied model is capable of correlating retention factors of the analytes in ternary solvent mobile phases. The dependence between the calculated and experimantal retention factors of the analytes is presented in Figure 1. A good agreement between theoretical and experimental values is observed.

Table 1. Retention factors (k) and the corresponding standard deviations (SD) of the investigated analytes in ternary solvent mobile phases of two different compositions (A and B)

A.

			Phenobarbital		Carbamazepine		Phenytoin	
f_1 (water)	f_2 (tetrahydrofuran)	f_3 (acetone)	k	SD	k	SD	k	SD
0.10	0.10	0.80	1.23	0.01	1.45	0.01	1.17	0.00[a]
0.10	0.20	0.70	1.18	0.00	1.52	0.00	1.06	0.01
0.10	0.30	0.60	1.06	0.00	1.40	0.00	1.00	0.01
0.10	0.40	0.50	1.06	0.01	1.41	0.00	1.06	0.01
0.10	0.50	0.40	1.06	0.01	1.24	0.02	1.06	0.01
0.10	0.60	0.30	1.18	0.00	1.06	0.01	1.17	0.00
0.10	0.70	0.20	1.23	0.01	1.17	0.00	1.17	0.00
0.10	0.80	0.10	1.18	0.00	1.17	0.00	1.09	0.01
0.20	0.10	0.70	–[b]	–[b]	1.36	0.00	–[b]	–[b]
0.20	0.20	0.60	1.42	0.00	1.34	0.00	–[b]	–[b]
0.20	0.30	0.50	1.06	0.01	1.00	0.00	0.93	0.00
0.20	0.40	0.40	1.06	0.01	1.00	0.00	1.02	0.00
0.20	0.50	0.30	1.17	0.00	1.06	0.02	1.08	0.01
0.20	0.60	0.20	1.17	0.00	1.12	0.01	2.02	0.00
0.20	0.70	0.10	1.12	0.01	[b]	[b]	[b]	[b]
0.30	0.20	0.50	–[b]	–[b]	1.35	0.03	–[b]	–[b]
0.30	0.50	0.20	1.80	0.02	1.80	0.01	–[b]	–[b]
0.30	0.60	0.10	–[b]	–[b]	3.36	0.00	3.70	0.00
0.40	0.10	0.50	3.94	0.01	3.92	0.01	5.04	0.00
0.40	0.20	0.40	3.60	0.02	3.38	0.00	4.59	0.02
0.40	0.30	0.30	3.38	0.02	3.04	0.00	4.26	0.01
0.40	0.40	0.20	3.41	0.02	2.84	0.01	4.38	0.00
0.40	0.50	0.10	3.94	0.01	3.15	0.01	3.72	0.00
0.50	0.10	0.40	4.16	0.02	4.17	0.01	4.61	0.01
0.50	0.20	0.30	3.92	0.01	3.49	0.01	4.36	0.00
0.50	0.30	0.20	3.02	0.00	2.48	0.01	3.70	0.00
0.50	0.40	0.10	–[b]	–[b]	2.48	0.01	2.70	0.00
0.60	0.10	0.30	2.80	0.01	3.25	0.01	3.46	0.01
0.60	0.20	0.20	3.14	0.01	2.70	0.00	–[b]	–[b]

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Table 1 (Continuation)

0.60	0.30	0.10	2.91	0.01	2.36	0.00	2.68	0.00
0.70	0.10	0.20	2.92	0.01	2.68	0.00	2.80	0.01
0.70	0.20	0.10	2.60	0.01	2.40	0.01	2.40	0.00
0.80	0.10	0.10	3.00	0.00	3.13	0.01	2.40	0.00

B.

			Phenobarbital		Carbamazepine		Phenytoin	
f_1 (water)	f_2 (methanol)	f_3 (acetonitrile)	k	SD	k	SD	k	SD
0.10	0.10	0.80	1.10	0.01	1.35	0.00	1.20	0.01
0.10	0.20	0.70	1.00	0.00	1.00	0.00	1.00	0.00
0.10	0.30	0.60	1.18	0.00	1.35	0.00	1.18	0.00
0.10	0.40	0.50	1.18	0.00	1.35	0.00	1.18	0.00
0.10	0.50	0.40	1.18	0.00	1.35	0.00	1.34	0.00
0.10	0.60	0.30	1.18	0.00	1.52	0.00	1.35	0.00
0.10	0.70	0.20	1.18	0.00	1.35	0.00	1.34	0.00
0.10	0.80	0.10	1.18	0.00	1.35	0.00	1.35	0.00
0.20	0.10	0.70	1.18	0.00	1.51	0.00	1.19	0.00
0.20	0.20	0.60	1.18	0.00	1.52	0.00	1.18	0.00
0.20	0.30	0.50	1.19	0.00	1.36	0.00	1.19	0.00
0.20	0.40	0.40	1.18	0.00	1.51	0.00	1.34	0.00
0.20	0.50	0.30	1.18	0.00	1.52	0.00	1.35	0.00
0.20	0.60	0.20	1.19	0.00	1.52	0.00	1.35	0.00
0.20	0.70	0.10	1.18	0.00	1.51	0.00	1.35	0.00
0.30	0.10	0.60	1.36	0.00	1.52	0.00	1.52	0.00
0.30	0.20	0.50	1.36	0.00	1.68	0.00	1.53	0.00
0.30	0.30	0.40	1.36	0.00	1.85	0.00	1.69	0.00
0.30	0.40	0.30	1.51	0.00	2.03	0.00	1.86	0.00
0.30	0.50	0.20	1.85	0.00	1.85	0.00	1.62	0.00
0.30	0.60	0.10	1.42	0.02	1.91	0.01	1.69	0.01

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Table 1 (Continuation)

0.33	0.33	0.34	1.51	0.01	1.87	0.01	1.60	0.04
0.40	0.10	0.50	1.51	0.00	1.93	0.01	1.77	0.01
0.40	0.20	0.40	1.76	0.02	2.77	0.01	2.47	0.01
0.40	0.30	0.30	1.58	0.01	1.92	0.01	2.14	0.01
0.40	0.50	0.10	1.35	0.00	2.58	0.01	2.15	0.00
0.50	0.10	0.40	1.69	0.00	2.23	0.01	2.25	0.02
0.50	0.40	0.10	2.53	0.01	3.08	0.01	2.90	0.00
0.60	0.10	0.30	2.90	0.00	3.46	0.00	2.90	0.00
0.60	0.20	0.20	2.90	0.00	3.46	0.00	2.90	0.00
0.60	0.30	0.10	2.96	0.00	2.93	0.00	2.96	0.02
0.70	0.10	0.20	3.00	0.00	3.53	0.00	2.93	0.00
0.70	0.20	0.10	2.93	0.00	3.50	0.00	3.50	0.00
0.80	0.10	0.10	2.60	0.01	3.10	0.01	3.50	0.00

[a] Aassumed if SD < 0.005.
[b] Not determined.

Table 2. Selected details concerning the investiagted sets: number of data points in each set (N), average percentage deviation (APD), correlation coefficients (R), and F values for the model (q = 1)

No.	Solvent system	Analyte	N	APD	R	F value[a]
1	Water + tetrahydrofuran – acetone	Phenobarbital	28	8.2	0.993	109.8
2		Phenytoin	27	7.6	0.995	140.0
3		Carbamazepine	30	8.4	0.992	103.8
4	Water – methanol – acetonitrile	Phenobarbital	34	6.3	0.987	78.8
5		Phenytoin	34	5.0	0.995	217.6
6		Carbamazepine	34	6.8	0.993	138.8
			Overall	7.0		
			S.D.	1.3		

[a] All F values were statistically significant (p < 0.0005).

The accuracy of the Jouyban–Acree model can be improved by appropriately changing the parameters (*i.e.* q values in Eq. (3)), as it is shown in Figure 2. More accurate calculations are achieve using higher q values. The best improvement is reached for q = 3 and higher q values produce the same accuracy as q = 3. One of the

aims of the modelling is to provide a practical and predictive tool to simulate the data. On the other hand, the more curve-fitting parameters are employed, the more accurate the calculations are obtained, see Figure 2. Although an optimum is obtained for q = 3, we prefer to assume q = 1; then, less experimental data points are required in the training step.

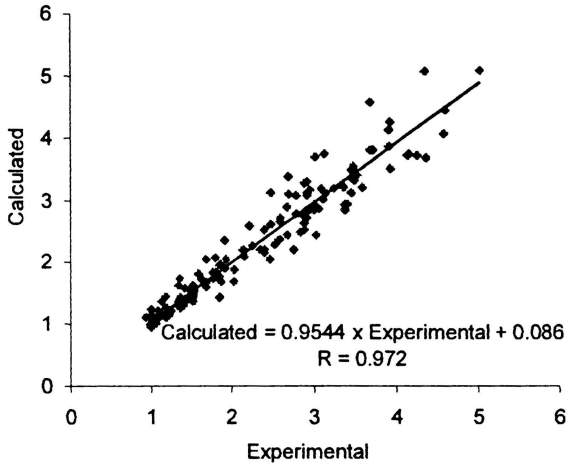

Figure 1. The calculated retention factors of the studied analytes in two ternary solvent mobile phases plotted *vs* the experimental values

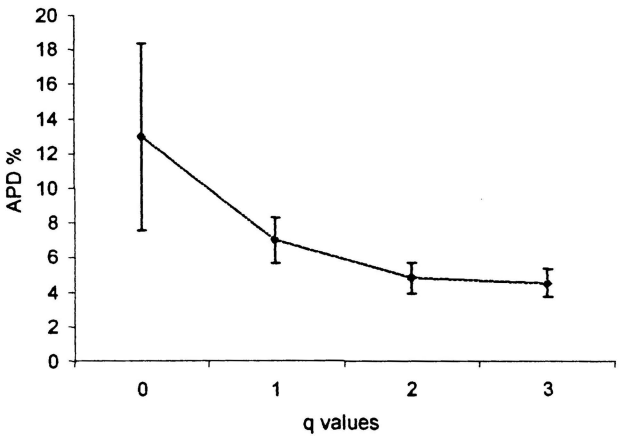

Figure 2. Average percentage deviation (APD) for the proposed model for different q values

With the proposed model, accurate k values are obtained for different solvent composition of the mobile phase. The model can be also used to speed up HPLC procedure when ternary solvent mobile phase is employed.

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