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Comparison of the Fitting Performance of Retention Models and Elution Strength Behaviour in Hydrophilic-Interaction and Reversed-Phase Liquid Chromatography

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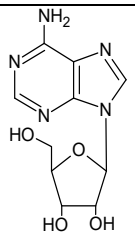
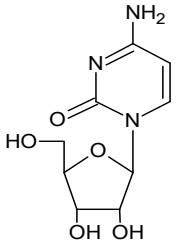
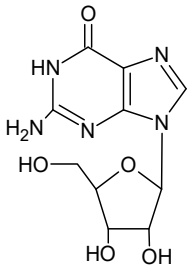
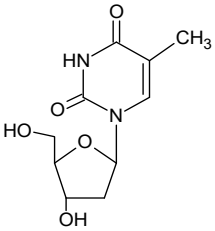
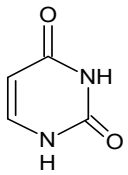
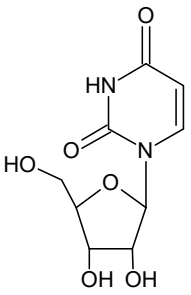
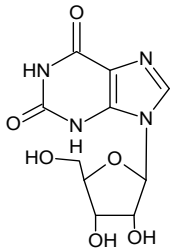
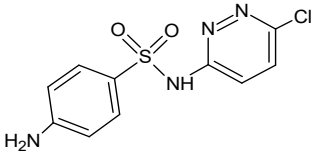
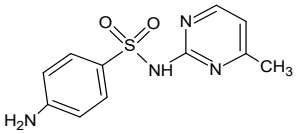
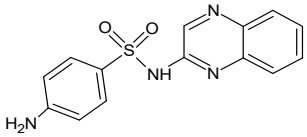
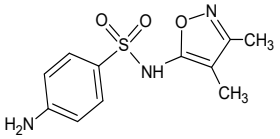
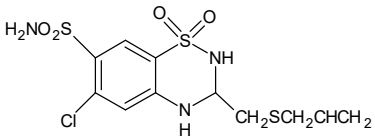
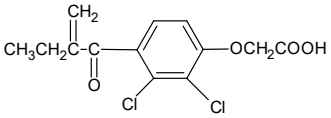
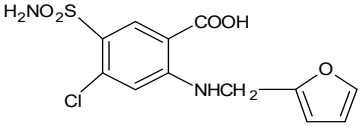
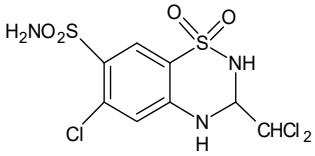
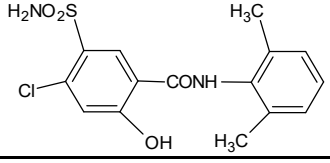
Table S1. Acidity constants (pK_a) and octanol-water partition coefficients (log $P_{o/w}$), for the probe compounds.

Compound	Structure	pK_a	log $P_{o/w}$
Adenosine		3.7[a]	–1.0 ± 0.5[b]
Cytidine		4.22; 12.5[a]	–1.9 ± 0.4[b]
Guanosine		2.1; 9.2[a]	–1.7 ± 0.6[b]
Thymidine		9.72[a]	–1.1 ± 0.5[b]
Uracil		9.5[a]	–0.7 ± 0.3[b]

Uridine		8.5[a]	–1.6 ± 0.4[b]
Xanthosine		5.5; 12.85[a]	–2.1 ± 0.6[b]
Sulphachloropyridazine		1.9, 5.1[c]	0.71[c]
Sulphamerazine		2.2, 7.0[c]	0.11[c]
Sulphaquinoxaline		5.5[c]	1.45[c]
Sulphisoxazole		1.8, 5.0[d]	0.81[c]
Althiazide		–	1.17[f]
Ethacrynic acid		3.5[e]	2.20[f]
Furosemide		7.5, 3.8[e]	1.81[f]
Trichloromethiazide		10.6, 8.6, 7.3[e]	1.00[f]
Xipamide		10.0, 4.8[e]	2.19[f]

[a] From Padivitage, N.L.; Dissanayake, M.K.; Armstrong, D.W. Separation of nucleotides by hydrophilic interaction chromatography using the FRULIC-N column, *Anal. Bioanal. Chem.* **2013**, *405*, 8837–8848. [b] Calculated with ChemSketch, ACD Labs, 2012 version, [c] From Carda-Broch, S.; Berthod, A. Countercurrent chromatography for the measurement of the hydrophobicity of sulfonamide amphoteric compounds, *Chromatographia* **2004**, *59*, 79–87. [d] From Białk-Bielinska, A.; Stolte, S.; Matzke, M.; Fabianska, A.; Maszkowska, J.; Kołodziejska, M.; Liberek, B.; Stepnowski, P.; Kumirska, J. Hydrolysis of sulphonamides in aqueous solutions, *J. Hazard Mater.* **2012**, *221–222*, 264–274. [e] From Hansch, C. In *Comprehensive Medicinal Chemistry*, Sammes, R.G.; Taylor, J.B., Eds. Pergamon Press: Oxford, 1990, vol. 6, and Ventura, R.; Segura, J. Detection of diuretic agents in doping control, *J. Chromatogr. B* **1996**, *687*, 127–144. [f] From Berthod, A.; Carda-Broch, S.; García-Alvarez-Coque, M.C. Hydrophobicity of ionizable compounds: a theoretical study and measurements of diuretic octanol-water partition coefficients by countercurrent chromatography, *Anal. Chem.* **1999**, *71*, 879–888.

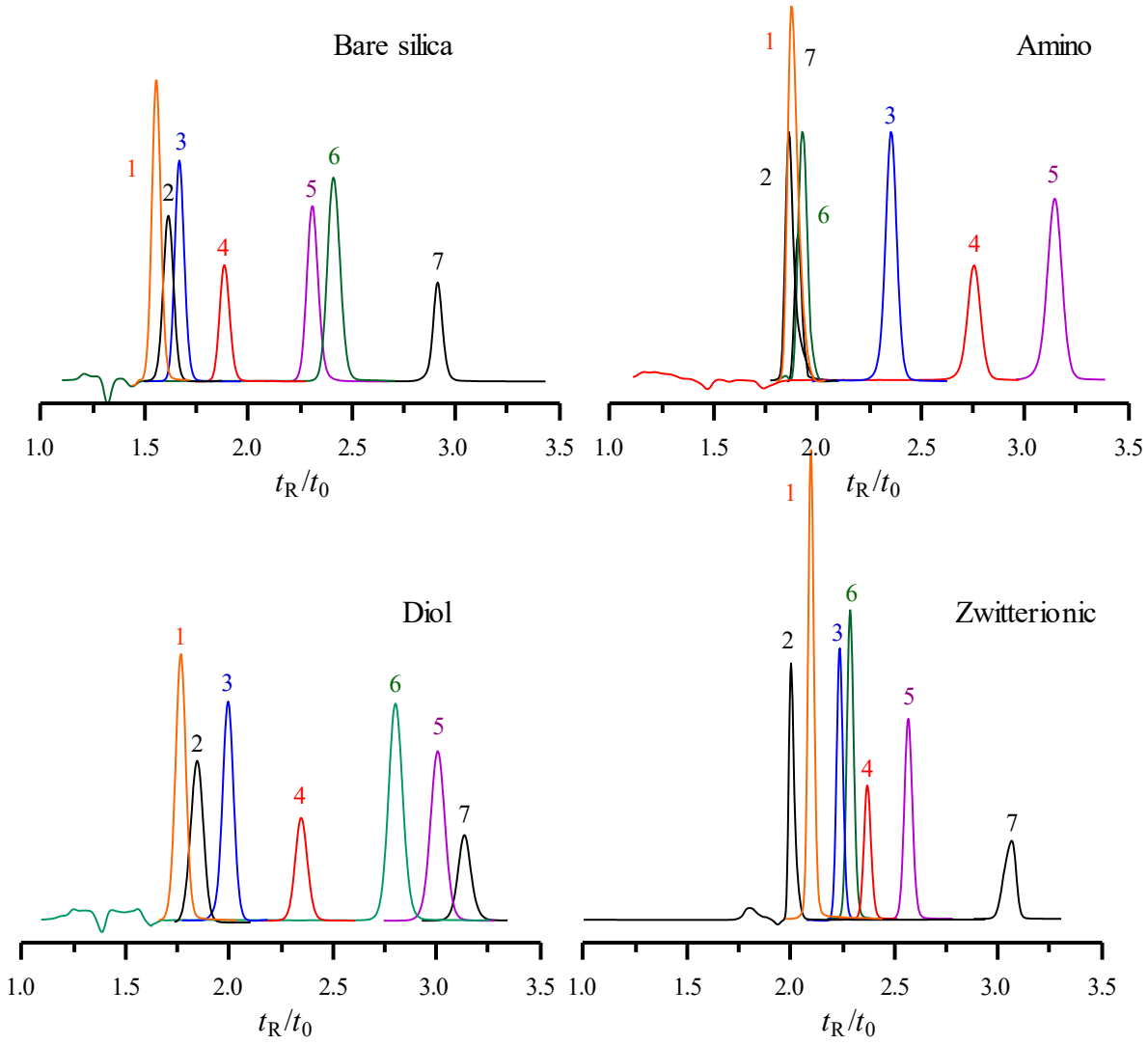


Figure S1. Chromatographic peaks obtained for uracil and six nucleosides eluted from HILIC columns, with a mobile phase containing 10 % water for bare silica, 11 % water for diol, 20 % water for amino, and 25 % water for the zwitterionic. The probe compounds (ordered according to their elution order with bare silica) are: (**1**) Uracil, (**2**) thymidine, (**3**) uridine, (**4**) xanthosine, (**5**) guanosine, (**6**) adenosine, and (**7**) cytidine.

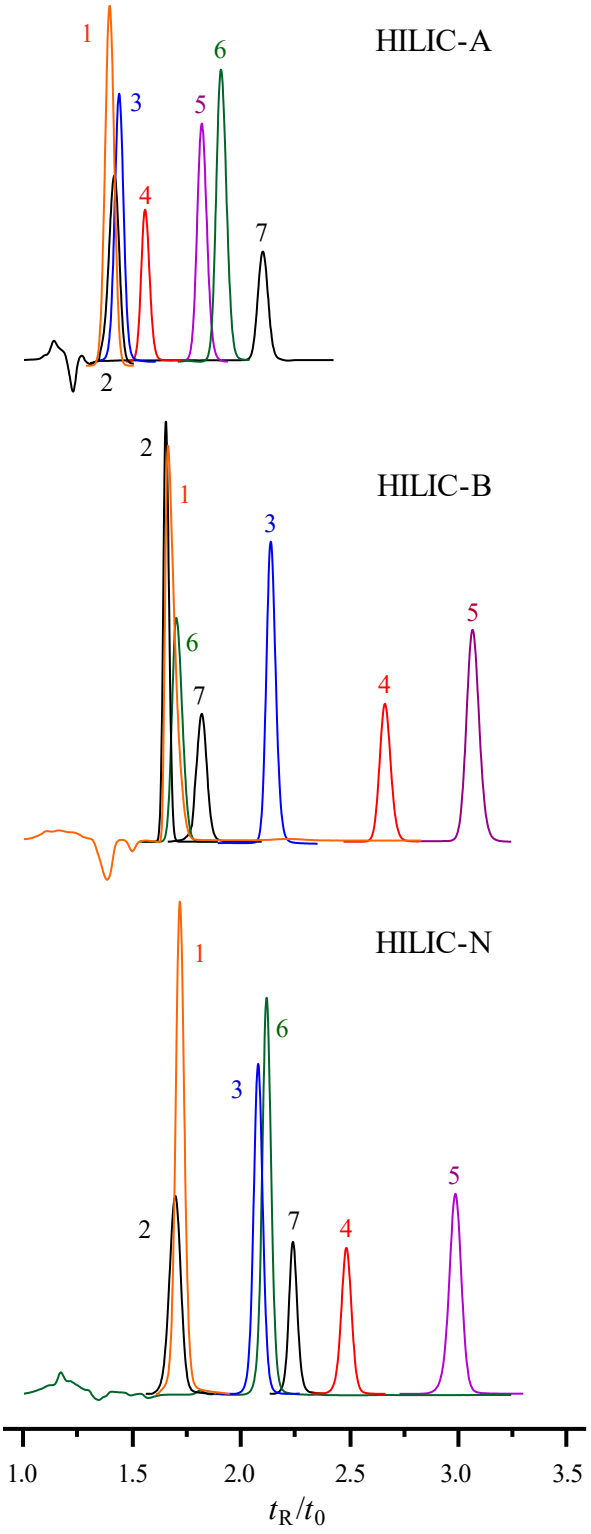


Figure S2. Chromatographic peaks obtained for uracil and six nucleosides eluted from HILIC columns, with a mobile phase containing 10 % water for HILIC A, and 20 % water for HILIC B and HILIC N. Other details are given in Figure S1.

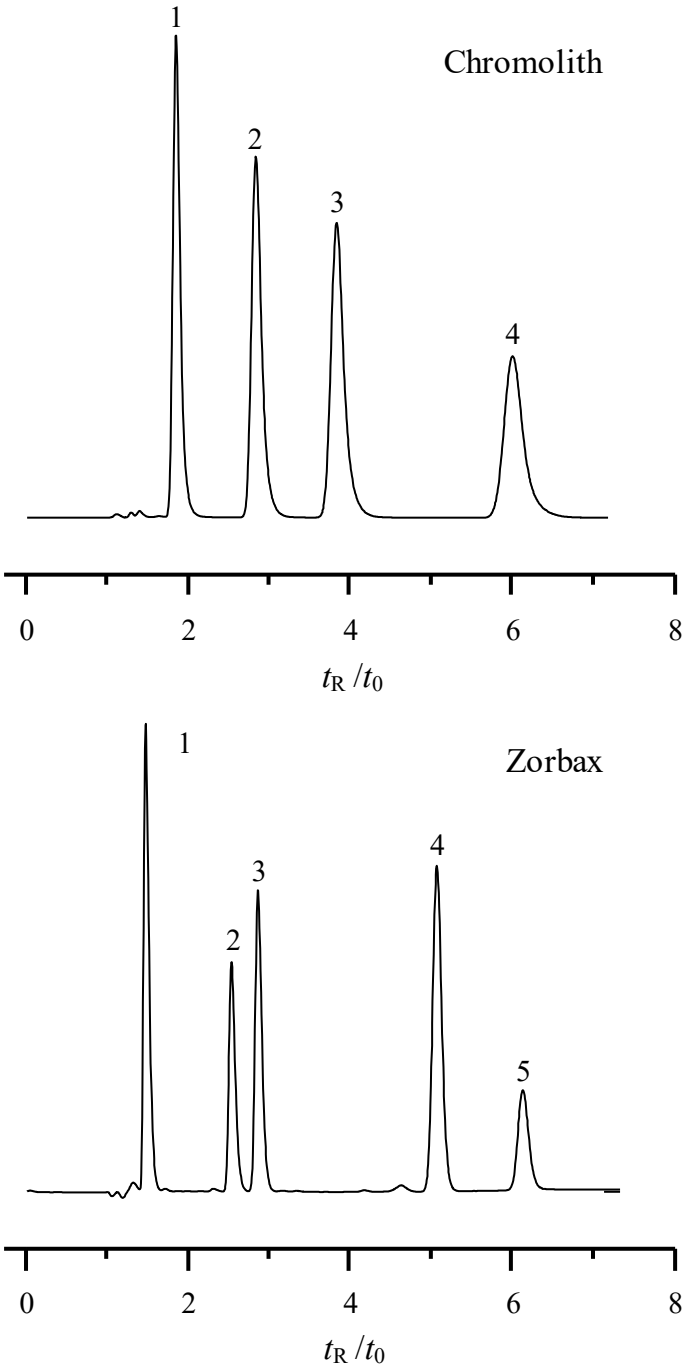


Figure S3. Chromatographic peaks obtained for sulphonamides and diuretics eluted from RPLC columns, with mobile phases containing 20% and 46% acetonitrile, respectively. Compound identities for Chromolith: (1) sulphamerazine, (2) sulphachloropyridazine, (3) sulphisoxazole, and (4) sulpha-quinoxaline. For Zorbax: (1) trichloromethiazide, (2) althiazide, (3) furosemide, (4) xipamide, and (5) ethacrynic acid.

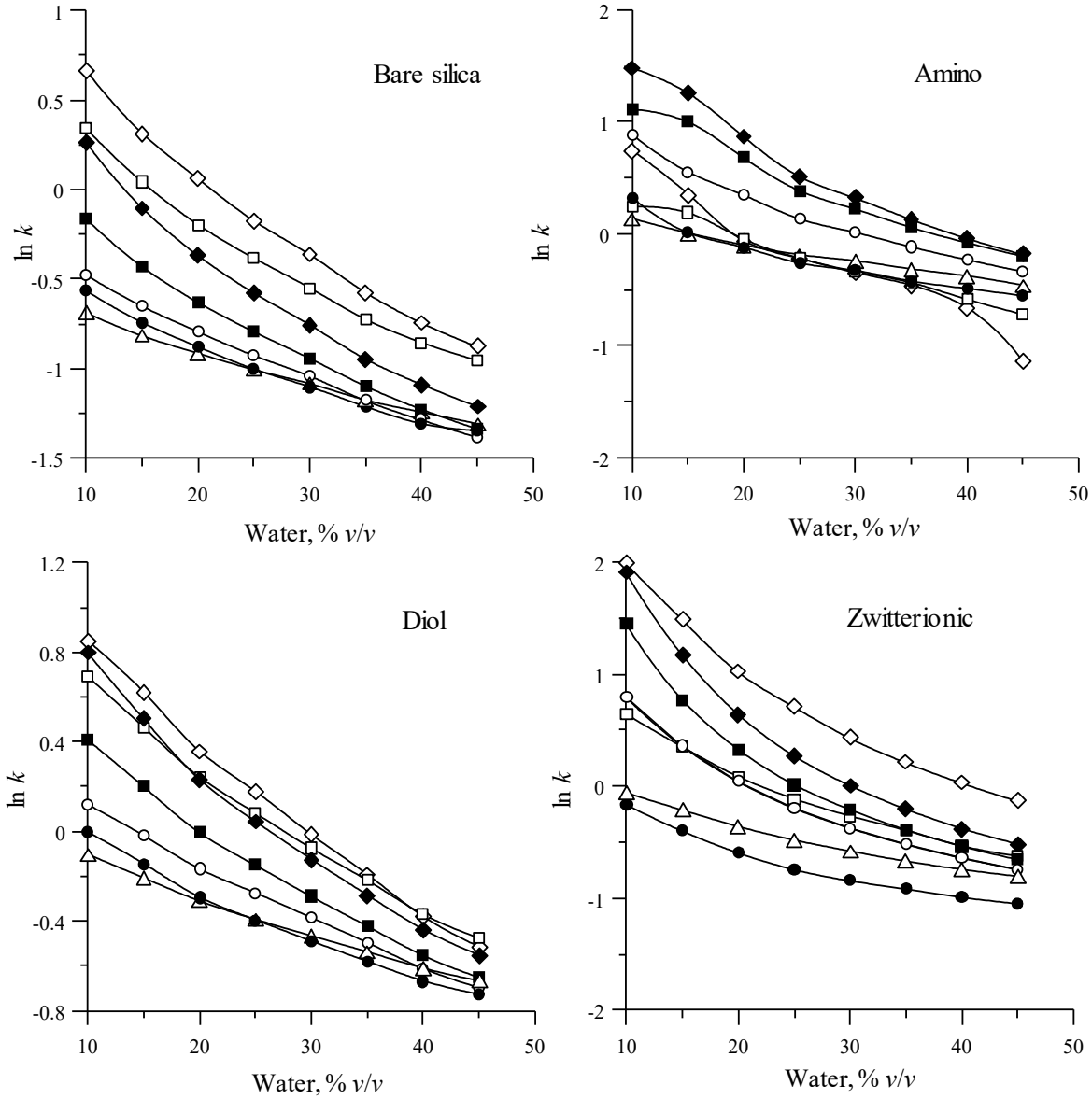


Figure S4. Retention behaviour for uracil and nucleosides, eluted with HILIC columns at increasing concentration of water. Compound identities (according to the elution order): Uracil (Δ), thymidine (●), uridine (○), xanthosine (■), guanosine (◆), adenosine (□), and cytidine (◊).

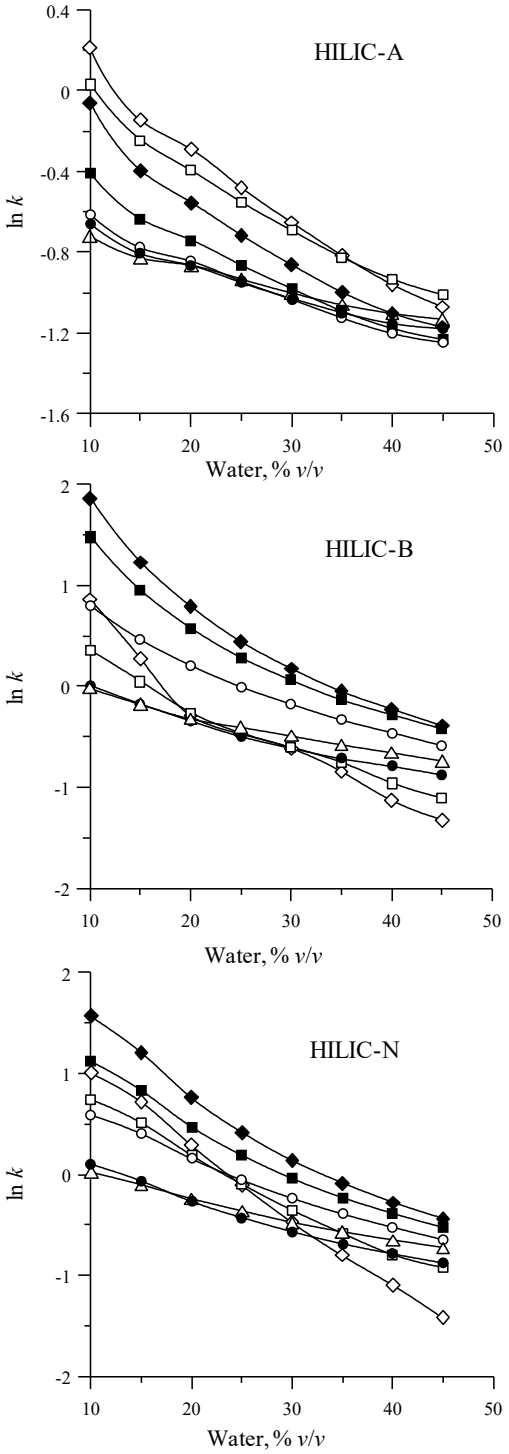


Figure S5. Retention behaviour for uracil and nucleosides, eluted with HILIC columns at increasing concentration of water. Compound identities are given in Fig. S4.

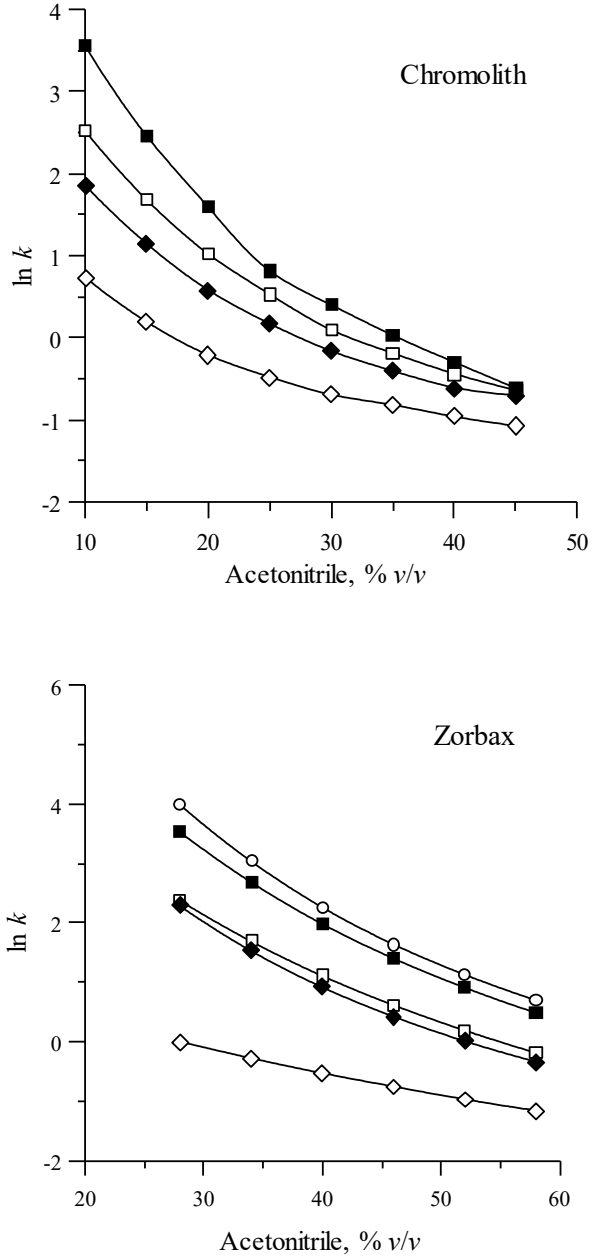


Figure S6. Retention behaviour for sulphonamides and diuretics, eluted with the RPLC columns at increasing concentration of acetonitrile. Compound identities for Chromolith: sulphamerazine (◊), sulphachloropyridazine (◆), sulphisoxazole (□), and sulphaquinoxaline (■), and for Zorbax: trichloromethiazide (◊), althiazide (◆), furosemide (□), xipamide (■), and ethacrynic acid (○).

Transformation of the original models to yield the modified equations

For Equation (S1) in main manuscript:

$$\ln k = \ln k_{\mathrm{w}} - S\varphi = \ln k_{\mathrm{w}} - S(\varphi_{\mathrm{o}} + \Delta\varphi) = \ln k_{\mathrm{w}} - S\varphi_{\mathrm{o}} - S\Delta\varphi = \ln k_{\mathrm{o}} - S\Delta\varphi \quad \text{(S1)}$$

For Equation (S3):

$$\ln k = \ln k_{\mathrm{w}} - a\varphi + b\varphi^2 = \ln k_{\mathrm{w}} - a(\varphi_{\mathrm{o}} + \Delta\varphi) + b(\varphi_{\mathrm{o}} + \Delta\varphi)^2 \quad \text{(S1)}$$

$$\ln k = \ln k_{\mathrm{w}} - a(\varphi_{\mathrm{o}} + \Delta\varphi) + b(\varphi_{\mathrm{o}}^2 + \Delta\varphi^2 + 2\varphi_{\mathrm{o}}\Delta\varphi)^2 \quad \text{(S2)}$$

$$\ln k = \ln k_{\mathrm{w}} - a\varphi_{\mathrm{o}} + b\varphi_{\mathrm{o}}^2 - a\Delta\varphi + 2b\varphi_{\mathrm{o}}\Delta\varphi + b\Delta\varphi^2 \quad \text{(S3)}$$

$$\ln k = \ln k_{\mathrm{o}} - (a - 2b\varphi_{\mathrm{o}})\Delta\varphi + b\Delta\varphi^2 = \ln k_{\mathrm{o}} - c\,\Delta\varphi + b\,\Delta\varphi^2 \quad \text{(S4)}$$

For Equation (S4):

$$\ln k = \ln k_{\mathrm{w}} - a\frac{\varphi_{\mathrm{o}} + \Delta\varphi}{1 + b(\varphi_{\mathrm{o}} + \Delta\varphi)} \quad \text{(S5)}$$

$$\ln k = \ln k_{\mathrm{w}} - a\frac{\varphi_{\mathrm{o}}}{1 + b\varphi_{\mathrm{o}}} + a\frac{\varphi_{\mathrm{o}}}{1 + b\varphi_{\mathrm{o}}} - a\frac{\varphi_{\mathrm{o}} + \Delta\varphi}{1 + b\varphi_{\mathrm{o}} + b\Delta\varphi} \quad \text{(S6)}$$

$$\ln k = \ln k_{\mathrm{o}} + a\frac{\varphi_{\mathrm{o}} + b\varphi_{\mathrm{o}}^2 + b\varphi_{\mathrm{o}}\Delta\varphi - \varphi_{\mathrm{o}} - \Delta\varphi - b\varphi_{\mathrm{o}}^2 - b\varphi_{\mathrm{o}}\Delta\varphi}{(1 + b\varphi_{\mathrm{o}})(1 + b\varphi_{\mathrm{o}} + b\Delta\varphi)} \quad \text{(S7)}$$

$$\ln k = \ln k_{\mathrm{o}} - a\frac{\Delta\varphi}{(1 + b\varphi_{\mathrm{o}})(1 + b\varphi_{\mathrm{o}} + b\Delta\varphi)} \quad \text{(S9)} \quad \text{(S8)}$$

$$\ln k = \ln k_{\mathrm{o}} - \frac{a}{1 + b\varphi_{\mathrm{o}}} \times \frac{\Delta\varphi}{1 + b\varphi_{\mathrm{o}} + b\Delta\varphi} \quad \text{(S10)} \quad \text{(S9)}$$

$$\ln k = \ln k_{\mathrm{o}} - \frac{\frac{a}{(1 + b\varphi_{\mathrm{o}})^2}\Delta\varphi}{1 + \frac{b}{1 + b\varphi_{\mathrm{o}}}\Delta\varphi} = \ln k_{\mathrm{o}} - c\frac{\Delta\varphi}{1 + d\,\Delta\varphi} \quad \text{(S11)} \quad \text{(S10)}$$

For Equation (S5):

$$k = \frac{(a\varphi_{\mathrm{o}})^{-m}}{(a\varphi_{\mathrm{o}})^{-m}}(a\varphi)^{-m} = e^{\ln k_{\mathrm{o}}}\left(\frac{a\varphi}{a\varphi_{\mathrm{o}}}\right)^{-m} = e^{\ln k_{\mathrm{o}}}\left(\frac{\varphi}{\varphi_{\mathrm{o}}}\right)^{-m} \quad \text{(S11)}$$

where the following was considered:

$$k_{\mathrm{o}} = e^{\ln k_{\mathrm{o}}} = (a\varphi_{\mathrm{o}})^{-a} \quad \text{(S12)}$$

For Equation (6):

$$k = (a + b(\varphi_{\mathrm{o}} + \Delta\varphi))^{-m} = (a + b\varphi_{\mathrm{o}} + b\Delta\varphi)^{-m} = (a_{\mathrm{o}} + b\Delta\varphi)^{-m} \quad \text{(S13)}$$

For Equation (S7):

$$k = \left(\frac{\varphi}{\varphi_o}\right)^{-m} \varphi_o^{-m} e^{a + b(\varphi_o + \Delta\varphi)} = \left(\frac{\varphi}{\varphi_o}\right)^{-m} e^{\ln k_o + b\Delta\varphi} \quad \text{(S14)}$$

where:

$$k_o = \varphi_o^{-m} e^{a + b\varphi_o} \quad \text{(S15)}$$

For Equation (S8):

$$k = (1 + b(\varphi_o + \Delta\varphi))^{-m} e^{a + c(\varphi_o + \Delta\varphi)} = (1 + b\varphi_o + b\Delta\varphi)^{-m} e^{a + c(\varphi_o + \Delta\varphi)} =$$
$$= \left(1 + \frac{b}{1 + b\varphi_o}\Delta\varphi\right)^{-m} (1 + b\varphi_o)^{-m} e^{a + c(\varphi_o + \Delta\varphi)} \quad \text{(S16)}$$

Since:

$$k_o = (1 + b\varphi_o)^{-m} e^{a + c\varphi_o} \quad \text{(S17)}$$

the following is obtained from Equation (S17):

$$k = (1 + d\,\Delta\varphi)^{-m} e^{\ln k_o + c\,\Delta\varphi} \quad \text{(S18)}$$

Table S2. Correlation of the parameters in the LSS model for the assayed HILIC and RPLC columns. Parameters and determination coefficient for the lines in Figures 1 and 2 ($S = n + m \ln k_o$) are given.

Column	*n*	*m*	R^2
Silica	3.78	2.29	0.923
Diol	2.13	2.57	0.969
Amino	2.17	2.38	0.706
Zwitterionic	2.98	3.49	0.883
HILIC-A	3.57	2.84	0.952
HILIC-B	3.06	3.39	0.787
HILIC-N	2.59	3.15	0.860
Chromolith	3.39	4.67	0.988
Zorbax	4.34	2.58	0.981

Table S3. Correlation of $S(\varphi)$ versus $\ln k_1$ (lowest value in the considered experimental range), for the assayed HILIC and RPLC columns. Parameters and determination coefficient for the lines in Figures 3 and 4 ($S = n + m \ln k_1$) are given.

Column	*n*	*m*	R^2
Silica	3.34	2.76	0.809
Diol	5.31	2.90	0.846
Amino	3.42	1.97	0.202
Zwitterionic	4.78	4.35	0.853
HILIC-A	5.59	3.97	0.735
HILIC-B	4.99	3.59	0.602
HILIC-N	3.86	1.66	0.432
Chromolith	6.78	4.90	0.968
Zorbax	6.87	3.24	0.962

Table S4. Fitting of the experimental retention data to the elution degree model (Equation (24)), for uracil and nucleosides, obtained in HILIC with different columns and water in acetonitrile as modifier. Model parameters, fitting determination coefficients, and relative errors of prediction are given.

	$\ln k_o$	$S_g \times 10^2$	g	R^2	E_r (%)
Underivatised silica					
Uracil	-0.69	5.69	2.15	0.9994	0.10
Cytidine	0.66	4.87	1.65	0.9996	0.46
Guanosine	0.26	6.69	1.86	0.9997	0.28
Adenosine	0.34	5.07	1.83	0.9998	0.24
Xanthosine	-0.16	6.56	1.86	0.9995	0.24
Uridine	-0.47	4.63	1.62	0.9996	0.14
Thymidine	-0.56	7.71	2.22	0.9991	0.18
Mean			1.88 ± 0.23		0.23 ± 0.12
Diol					
Uracil	-0.105	2.62	2.22	0.9998	0.10
Cytidine	0.85	3.74	1.39	0.9992	0.56
Guanosine	0.80	3.69	1.79	0.9997	0.34
Adenosine	0.69	3.19	1.67	0.9996	0.32
Xanthosine	0.41	3.43	1.78	0.9996	0.27
Uridine	0.12	2.78	1.57	0.9994	0.22
Thymidine	-0.003	3.38	2.21	0.9993	0.21
Mean			1.80 ± 0.30		0.29 ± 0.14
Amino					
Uracil	0.13	2.23	2.72	0.9977	0.37
Cytidine	0.74	5.25	1.90	0.988	2.47
Guanosine	1.53	4.14	1.37	0.9964	2.03
Adenosine	0.28	2.99	1.18	0.987	1.30
Xanthosine	1.39	3.19	1.75	0.9984	1.09
Uridine	0.88	2.66	2.24	0.9995	0.44
Thymidine	0.32	3.88	3.53	0.9981	0.47
Mean			2.10 ± 0.80		1.2 ± 0.8
HILIC-N					
Uracil	0.03	2.97	1.80	0.9990	0.28
Cytidine	1.03	7.31	0.98	0.9982	1.44
Guanosine	1.58	5.29	1.33	0.9966	2.33
Adenosine	0.76	5.20	1.14	0.9968	1.33
Xanthosine	1.13	4.54	1.42	0.9968	1.66
Uridine	0.60	3.76	1.40	0.9972	0.94
Thymidine	0.11	3.91	1.72	0.9979	0.52
Mean			1.30 ± 0.60		1.2 ± 0.7
HILIC-A					
Uracil	-0.73	6.90	2.86	0.9925	0.28
Cytidine	0.21	5.68	1.96	0.996	0.94
Guanosine	-0.065	8.30	2.42	0.9972	0.57
Adenosine	0.026	5.55	2.17	0.9981	0.47
Xanthosine	-0.42	7.94	2.42	0.9961	0.42
Uridine	-0.62	6.30	2.31	0.9945	0.36
Thymidine	-0.66	11.84	3.17	0.9941	0.32
Mean			2.50 ± 0.40		0.48 ± 0.22
HILIC-B					
Uracil	-0.020	3.65	2.49	0.9984	0.29
Cytidine	0.86	8.00	1.78	0.9984	1.11
Guanosine	1.86	4.33	1.71	0.99996	0.30
Adenosine	0.36	5.68	1.77	0.9981	0.80

Xanthosine	1.48	3.91	1.83	0.99995	0.28
Uridine	0.80	3.82	1.91	0.99998	0.10
Thymidine	0.013	4.43	2.12	0.9992	0.32
Mean			1.90 ± 0.30		0.5 ± 0.4
Zwitterionic					
Uracil	-0.055	4.04	2.31	0.9995	0.19
Cytidine	2.00	4.01	1.56	0.9991	1.35
Guanosine	1.91	5.14	1.71	0.9996	1.08
Adenosine	0.64	3.88	1.95	0.9994	0.46
Xanthosine	1.45	5.12	1.91	0.9998	0.62
Uridine	0.80	4.93	2.06	0.9998	0.36
Thymidine	-0.16	8.61	2.80	0.9985	0.35
Mean			2.00 ± 0.40		0.6 ± 0.4
Global mean			1.90 ± 0.60		0.7 ± 0.6

Table S5. Fitting of the experimental retention data to the elution degree model (Equation (24)), for sulphonamides and diuretics, obtained in RPLC with the Chromolith and Zorbax columns and acetonitrile in water as modifier. Model parameters, fitting determination coefficients and prediction relative errors are given.

	$\ln k_o$	$S_g \times 10^2$	g	R^2	E_r (%)
Chromolith					
Sulphamerazine	0.73	7.30	1.98	0.9993	0.77
Sulphachloropyridazine	1.86	6.52	1.55	0.9995	1.21
Sulphisoxazole	2.52	7.13	1.42	0.9998	1.03
Sulphaquinoxaline	3.56	9.47	1.28	0.9998	1.49
Mean			1.60 ± 0.30		1.1 ± 0.3
Zorbax					
Trichlormethiazide	-0.0091	4.91	1.40	0.99999	0.041
Althiazide	2.30	6.58	1.35	0.99998	0.25
Furosemide	2.39	6.49	1.28	0.99999	0.17
Xipamide	3.55	6.22	1.27	0.99999	0.28
Ethacrynic acid	4.00	6.20	1.27	0.99998	0.42
Mean			1.32 ± 0.06		0.19 ± 0.11
Global mean			1.40 ± 0.20		0.7 ± 0.5