Journal of Chromatography A, 1228 (2012) 125–134

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Hydrosilated silica-based columns: The effects of mobile phase and temperature on dual hydrophilic-reversed-phase separation mechanism of phenolic acids

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ARTICLE INFO

Article history:
Available online 30 June 2011

Keywords:
Hydrosilated silica
HILIC
Temperature effects
Mobile phase
Phenolic acids

ABSTRACT

The effects of mobile phase composition and of temperature on the retention behavior of phenolic acids were studied on 4 hydrosilated (type C silica) based columns in buffered aqueous acetonitrile, both in the aqueous normal phase (HILIC) and in the reversed-phase mobile phase range. The UDC cholesterol and the C_{18} bidentate columns show significant reversed phase and normal-phase retention mechanisms, whereas very weak retention in the reversed-phase mode was observed on the silica hydride and the Diamond hydride columns. The concentration effects of the aqueous acetate buffer over the full mobile phase (HILIC and RP) composition range can be described by a simple four-parameter equation. At increasing temperature, the retention times and peak widths decrease both in the aqueous normal phase and in the reversed phase mobile phase range. Linear van't Hoff log k versus $1/T$ plots were observed, indicating a single retention mechanism predominating in the highly organic (HILIC), like in highly aqueous (RP) mobile phase ranges. Besides the type of the stationary phase, the separation selectivity of phenolic acids strongly depends on temperature and on the mobile phase composition. From among the 4 hydrosilated columns compared in this work, the UDC cholesterol column has high temperature stability (up to 100 °C) and is most suitable for selective and efficient separations of phenolic acids both in the HILIC and in the RP modes.

1. Introduction

Hydrosilated silica gel (type C silica, silica hydride) was introduced many years ago. However, its interesting chromatographic properties have only recently found increasing use. The hydrosilation process changes significantly the properties of the silica gel surface by substituting up to 95% of original silanol (Si–O–H) groups from the surface of the adsorbent by less polar silicon hydride (Si–H) groups [1]. It can be used in normal-phase liquid chromatography with organic mobile phases [2], however its main application range is in aqueous normal phase LC.

The aqueous normal-phase LC mode (by many authors called "Hydrophilic Liquid Chromatography", HILIC) employs polar stationary phases in combination with mobile phases containing high concentrations of organic solvents (usually acetonitrile) in water, often with a buffer additive [3]. In the past years, HILIC technique has attracted attention as perspective complementary alternative to reversed-phase HPLC for separations of polar compounds, which still represent challenging problem [4–10]. Particularly interesting are HILIC applications for the analysis of peptides and other biopolymers [11–13] and in pharmaceutical [14] or metabolite analysis [15–17].

In aqueous–organic mobile phases, water is preferentially adsorbed on the surface of silica and other polar adsorbents; consequently, a diffuse water-rich layer forms on the adsorbent surface. In the HILIC range, polar compounds may be retained due to combined adsorption on the adsorbent surface and partition into the diffuse adsorbed aqueous layer. Ion-exchange interactions with charged functional groups may contribute to the retention of ionic or partly ionized samples, so that the resulting HILIC mechanism may be quite complex. Hydrosilated silica gel surface shows less attraction for water molecules with respect to the ordinary silica gel type B. Due to its more hydrophobic surface, hydrosilated silica is believed to form less dense adsorbed water layer at its surface than other, more polar stationary phases employed for HILIC separations [18–24]. The hydride surface of hydrosilated silica is slightly hydrophobic and can retain some weakly polar compounds in highly aqueous mobile phases in the reversed-phase mode, even though much less strongly than common C_{18} or C_8 alkylsilica stationary phases. To increase the retention of hydrophilic compounds under reversed-phase conditions, the hydrophobicity of the silica hydride surface was enhanced by chemical modification introducing low-polarity bonded groups, which also provide some new selectivity properties for separations of polar compounds. Fig. 1 shows schematically the surface structure of hydrosilated silica and

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doi:10.1016/j.chroma.2011.06.077

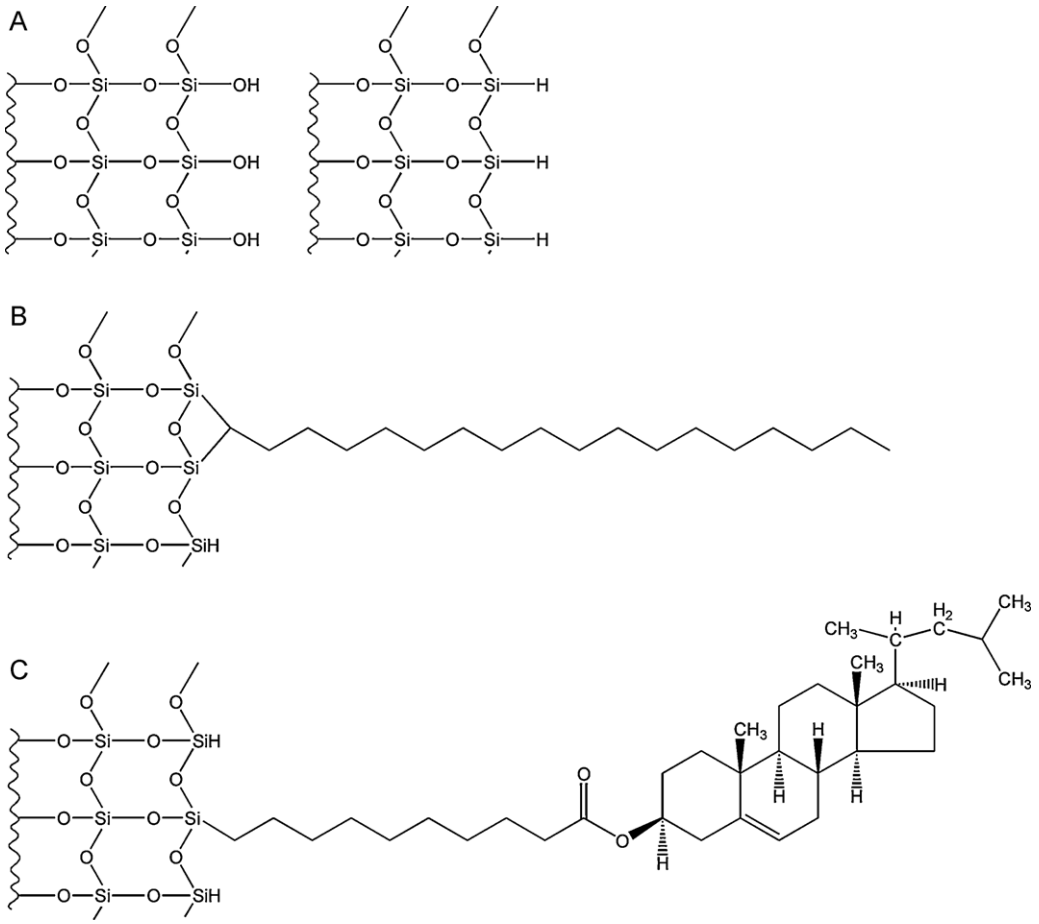

Fig. 1. Structures of stationary phases. (A) Silica type B and silica hydride, type C, (B) – C18 bidentate, (C) – UDC cholesterol.

ordinary silica (A) and of hydrosilated silica modified with bidentate C_{18} (B) and cholesterol (C) groups. Silica hydride modified with undecanoic acid (UDA silica) is also commercially available. Diamond hydride stationary phases, which contain ca 2.5% carbon, show improved HILIC separation selectivity for mono-, di- and tri-phosphate nucleotides, due to enhanced ion-interaction (ion repulsion) properties with respect to the Diamond hydride column [24].

Silica hydride columns modified with non-polar moieties show some features of dual reversed-phase/normal-phase retention mechanism and can be used either in highly aqueous mobile phases in the RP mode, or for separations in the aqueous normal-phase (HILIC) mode in buffered mobile phases containing more than 50–70% acetonitrile [25], unlike the un-modified silica hydride column, which shows very low hydrophobic selectivity and retention under RP conditions [26].

In normal-phase chromatography (NP), the retention increases with increasing sample polarity. The mobile phase affects significantly the retention in LC and in classical non-aqueous adsorption NP chromatography the retention decreases as the concentration of a polar solvent with high elution strength increases in binary organic mobile phases. Likely, at high concentrations of the organic solvent in the HILIC mobile phase range, the retention on polar stationary phases decreases at increasing concentration of water as the more polar solvent in aqueous–organic mobile phases. On the same polar column, the retention may decrease for more polar samples and at increasing concentrations of organic solvent in highly aqueous binary mobile phases, showing typical RP behavior. Consequently, the graphs displaying the effects of the composition of aqueous–organic mobile phases on the retention show characteristic "U shape". Assuming additivity of the HILIC and RP contributions to the retention, the effect of the volume fraction of water (or of an aqueous buffer), $\varphi(H_2O)$, on the retention factors, k, in the full composition range of aqueous–organic mobile phases can be described, to first approximation, by Eq. (1) [27,28]:

$$\log k = a_1 + m_{RP}\varphi(H_2O) - m_{HILIC}\log\varphi(H_2O) \quad (1)$$

The parameter m_{HILIC} characterizes the effect of the aqueous component on the rate of decreasing HILIC contribution to the retention in highly organic mobile phases, while the parameter m_{RP} describes its effect on the rate of increasing contribution of the RP mechanism to the retention in the aqueous-rich mobile phases; a is an empirical constant and has no exact physical meaning. The parameters a_1, m_{RP} and m_{HILIC} can be determined by non-linear regression of the experimental retention factors measured at varying volume fractions of water (or of aqueous buffer) in the mobile phase [29]. Eq. (1) applies in HILIC systems only if the sample is very strongly retained in 100% acetonitrile, otherwise Eq. (2) often offers better approach to the description of the dual HILIC/RP retention mechanism [30]:

$$\log k = a_2 + m_{RP}\varphi(H_2O) - m_{HILIC}\log[1 + b\varphi(H_2O)] \quad (2)$$

m_{RP} and m_{HILIC} have similar meaning as in Eq. (1); the parameter b is the correction term for limited HILIC retention in mobile phases with very low concentrations of water.

The potential role of temperature effects in HPLC method development has not been yet fully recognized, obviously because of a limited temperature stability of the ordinary stationary phases chemically bonded on the silica gel type B support (often only up to 60 °C). However, bare silica and some stationary phases bonded on hydrosilated silica surface show enhanced temperature stabil-

Table 1
Characteristics of the hydrosilated columns employed. A: Silica hydride, B: C_{18} bidentate, C: UDC cholesterol, D: Diamond hydride *L*: column length, I.d.: internal diameter, V_M: hold-up volume measured with toluene in 95% acetonitrile ($V_{M,T}$: HILIC mode) and with uracil in 15% acetonitrile ($V_{M,U}$: RP mode), ε_T: total porosity in the HILIC ($\varepsilon_{T,T}$) and in the RP ($\varepsilon_{T,U}$) modes, n_T: number of theoretical plates for toluene in the HILIC mode (0.5 mL min^{-1}), n_U: number of theoretical plates for uracil in the reversed-phase mode (0.5 mL min^{-1}), V_S/V_M: column phase ratio – Eq. (4), and T_{MAX}: column temperature stability limit, according to the manufacturer's data.

Column	*L* (mm)	i.d. (mm)	$V_{M,T}$ (mL) (HILIC)	$V_{M,U}$ (mL) (RP)	$\varepsilon_{T,T}$	$\varepsilon_{T,U}$	pH range	T_{MAX} (°C)	n_T	n_U	Particle size (μm)	V_S/V_M (HILIC)	V_S/V_M (RP)
A	75	4.6	0.57	0.59	0.46	0.47	2.0–7.0	100	1759	1558	4	1.17	1.13
B	75	4.6	0.52	0.59	0.42	0.47	2.0–9.2	80	1611	1827	4	1.38	1.13
C	75	4.6	0.54	0.54	0.43	0.43	2.0–8.0	100	1922	2062	4	1.33	1.33
D	100	4.6	1.04	1.04	0.63	0.63	2.5–7.0	60	3320	3844	4	0.60	0.60

ity range. For these columns, the control of temperature has some advantages, as it can be easily adjusted in the instrument equipped with a thermostatted column compartment. In most RP and HILIC separation systems an increase of temperature causes a decrease in retention. Solvent viscosity decreases at higher temperature, causing diffusion coefficients to increase, which often improves the efficiency of separation (column plate number) and peak shape [31]. Further, the column backpressure decreases at increased temperature, so that higher flow rates can be used for faster separations [32]. Temperature often affects chromatographic selectivity, especially for ionizable compounds such as weakly acidic phenolic compounds, as the ionization equilibria usually can be shifted by a change in temperature.

The effects of thermodynamic temperature, *T* (in Kelvin) on the sample retention factors, *k*, is described by van't Hoff equation (Eq. (3)) [33–36]:

$$\log\ k = \log\ K + \log\ \frac{V_S}{V_M} = \frac{-\Delta G^0}{RT} + \log\ \frac{V_S}{V_M} = \frac{\Delta S^0}{R} + \log\ \frac{V_S}{V_M} - \frac{\Delta H^0}{RT} = A_i + \left(\frac{B_i}{T}\right) \quad (3)$$

According to Eq. (3), the $\log k$ versus $1/T$ plots should be linear, the parameter B_i being proportional to the standard partial molar enthalpy of transfer of the solute *i* from the mobile phase to the stationary phase, $-\Delta H^0$; the parameter A_i includes the change in the standard partial molar entropy connected with the transfer of the solute from the mobile phase to the stationary phase, ΔS^0, and the phase ratio (the ratio of the volumes of the stationary, V_S, and of the mobile, V_M, phases) in the chromatographic system. *R* is the gas constant. Possible deviations of the experimental data from the linear Eq. (3) may indicate changing retention mechanism in the investigated temperature range [36–40]. Hearn and Zhao [41] observed non-linear $\log k$ versus $1/T$ plots for several polypeptides in acetonitrile–water mobile phases on an alkylsilica stationary phase and explained it by temperature effects on changing heat capacity, so that the entropy of retention S^0 depends on temperature. The intercept term, A_i, includes the contribution of the column phase ratio, which is assumed to be independent of temperature; however Guillarme et al. [42] attributed some deviations from the linearity of the $\log k$ versus $1/T$ experimental plots to possible changes in the phase ratio caused by the temperature effects on the system backpressure.

From the experimental data set measured over a sufficiently broad temperature range, one may calculate the enthalpic contribution to the retention and selectivity, $-\Delta H^0$, from the slope, B_i, and the entropic contribution, ΔS^0 from the intercept, A_i, of the $\log k$ versus $1/T$plots. For calculation of the entropic contribution, the numerical value of the phase ratio in the column should be known, which may not be easy to determine because of difficulties with clear definition of the boundary between the region occupied by the stationary and by the mobile phase in the column [43]. For this purpose, a simplified convention (even though not exact) can be accepted, defining the volume of the stationary phase, V_S, as the part of the total column volume, V_C, into which non-retained compounds cannot penetrate. With this convention, the phase ratio can be calculated from the total column porosity, $\varepsilon_T = V_M/V_C$ [44,45]:

$$\frac{V_S}{V_M} = \frac{V_C - V_M}{V_M} = \frac{1 - \varepsilon_T}{\varepsilon_T} \quad (4)$$

HILIC methods are suitable for separation of phenolic acids in plants, fruit and vegetables [29,30]. The interest in the analysis of phenolic acids is continuously increasing, due to the protection antioxidant role of many phenolic compounds in human body against cancer and coronary heart diseases. The objective of the present work was investigating the effects of mobile phase and temperature on the retention, selectivity and resolution of phenolic acids and to compare possibilities of their separation on various hydrosilated silica-based columns in the HILIC and in the reversed-phase (RP) modes.

2. Experimental

2.1. Equipment

All the experiments were measured with an HP 1090 (Agilent, Palo Alto, CA, USA) liquid chromatograph equipped with a UV diode array detector. The columns were placed in a thermostatted column compartment and the detection wavelength was set to 275 nm, the UV absorption maximum for phenolic acids.

2.2. Materials and reagents

The characteristics of the Cogent hydrosilated silica gel columns (all from MicroSolv, Eatontown, NJ, USA), are listed in Table 1; the structures of the stationary phase surface are shown in Fig. 1:

A a Cogent Silica Hydride column, 75 × 4.6 mm id, 5 μm, obtained as a gift from Prof. J. Pesek (San Jose University, CA, USA),
B a Cogent Bidentate C18 column (75 × 4.6 mm id, 5 μm),
C a Cogent UDC cholesterol column, 75 × 4.6 mm id, particle size 5 μm,
D a Cogent Diamond Hydride column, 100 × 4.6 mm id, particle size 4 μm (not shown).

The standards of phenolic acids were purchased from Sigma–Aldrich in the best available purity; the structures are shown in Fig. 2. Acetonitrile (LiChrosolv grade), ammonium acetate and formic acid (both reagent grade) were obtained from Merck, Darmstadt, Germany. Water was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.3. Methods

The mobile phases were prepared by mixing appropriate volumes of 10 mmol/L solution of CH_3COONH_4 in water (with pH adjusted to 3.26 by addition of a few drops of HCOOH) in water

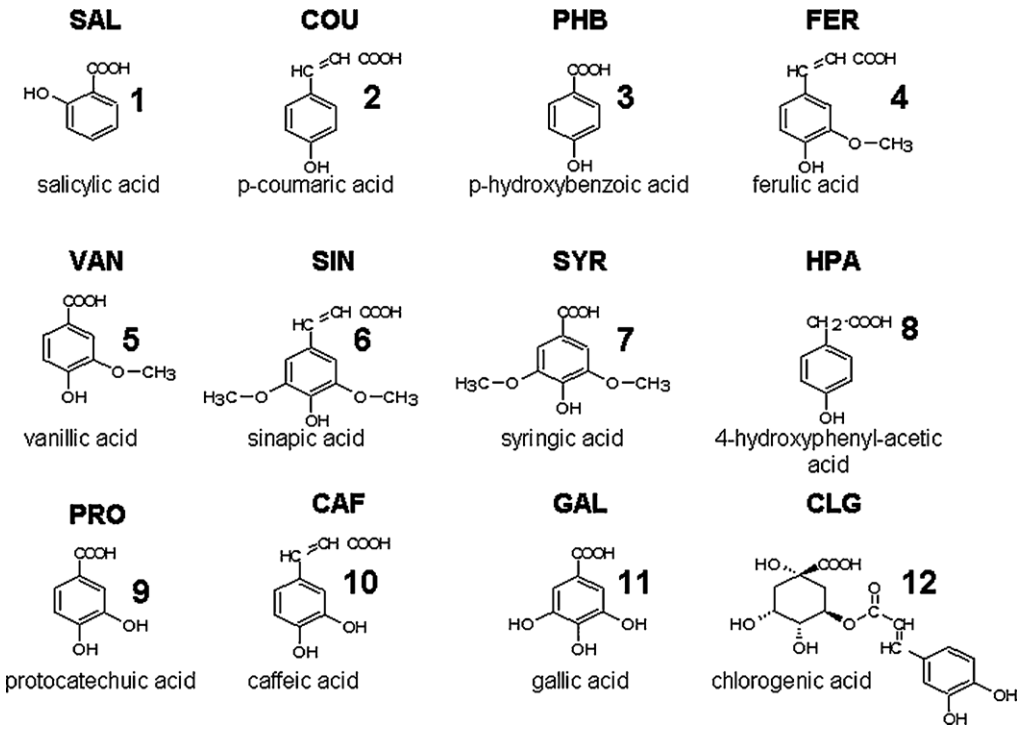

Fig. 2. Structures of the phenolic acid standards.

with 10 mmol/L solution of CH_3COONH_4 in acetonitrile to suppress the ionization of weak phenolic acids. The stock solutions of phenolic acid standards were prepared in 95% aqueous acetonitrile and working solutions were obtained by appropriate diluting the stock solutions in the mobile phase. The column hold-up volume, V_M, was determined as the elution volume of toluene in the HILIC mode ($V_{M,T}$) and of uracil in the RP mode ($V_{M,U}$) as the non-retained markers (Table 1). Before each new series of experiments, the columns were equilibrated by flushing with 20 column hold-up volumes of the fresh mobile phase and the separation temperature was adjusted. The retention times, t_R, were measured over the full composition range of the mobile phases containing 10 mmol/L ammonium acetate in aqueous acetonitrile. The measurements were repeated in triplicate and arithmetic means of the experimental retention times, t_R, and the appropriate column hold-up time, $t_{M,T}$, or $t_{M,U}$, were used to calculate the retention factors, $k = t_R/t_M - 1$. The Adstat 1.25 software (Trilobyte Statistical Software, Pardubice, Czech Republic) was utilized for the determination of the parameters of Eqs. (1)–(3) by either linear or non-linear regression of the experimental data sets.

3. Results and discussion

3.1. Mobile phase and structural effects on the retention in the HILIC and in the RP range

The dimensions and other characteristics of the hydrosilated columns tested are listed in Table 1; Fig. 1 shows schematically their surface structure (the manufacturer's data). The Silica hydride material (silica gel C) is prepared by hydrosilation reaction, in which the silica hydride groups (Si–H) may replace up to 95% of the silanols on the surface of silica gel A (Fig. 1A) The alkyl ligands in the Cogent Bidentate C_{18} column are bonded directly onto the silica surface with two separate points of attachment (Fig. 1B), according to the manufacturer's information the bonded phase is stable in the pH interval from 2.0 to 9.2, up to 80 °C. The Cogent UDC cholesterol column (Fig. 1C) is claimed to be stable in the pH range from 2.0 to 8.0 and up to 100 °C. A Cogent Diamond Hydride column (proprietary surface structure, not shown), has lower thermal stability, up to 60 °C, and working pH range from 2.5 to 7.0.

Structural characteristics (solvatochromic parameters), characterizing the selective interaction effects on the solubility and retention, of the phenolic acids tested, are listed in Table 2. The Abraham Linear Structure-Energy Relationship (LSER) model, which employs multiple correlations between the retention ($\log k$) and the solvatochromic parameters [46–48]:

$$\log\ k = \log\ k_0 + \frac{m' V_X}{100} + s'\pi + a'\alpha + b'\beta \quad (5)$$

Eq. (5) allows correlating the retention of different solutes on the same column and in the same mobile phase with solute properties. The solvatochromic parameters of Eq. (5) include molecular structural descriptors characterizing the sample: the volume of solvated solute V_X, permanent and induced dipole–dipole polar interactions π, hydrogen bonding basicity β, and hydrogen bonding acidity α. The coefficients m', s', a' and b' of Eq. (5) provide a measure of the response of the system stationary/mobile phase to the selective properties of analytes. We applied the LSER approach for the comparison of the retention and separation selectivity of phenolic acids on the hydrosilated silica columns in the high-organic (HILIC) and low-organic (RP) range of buffered acetonitrile–water mobile phases.

All tested columns can be used for normal-phase (HILIC) separations of less polar phenolic acids with a single phenolic –OH group in mobile phases containing more than 85% acetonitrile. Protocatechuic, caffeic, gallic and chlorogenic acids with 2 or 3 phenolic groups (Nos. 9, 10, 11 and 12 in Fig. 2) are too strongly retained in the HILIC mode and show very asymmetric peaks. The peak symmetry did not improve significantly when changing the pH of the mobile phase, or when varying the ammonium acetate buffer ionic strength in between 5 mmol/L and 20 mmol/L. We used the columns at the upper temperature limits indicated in the manufacturer's literature and we did not observe any separation deterioration over the two-months working period.

Table 2
Solute solvatochromic parameter descriptors (Eq. (5)) and pK_a of the phenolic acids studied.

No.	Compound	pK_a	V_X	π	α	β
1	SAL	2.97	0.990	0.85	0.73	0.37
2	COU	3.70	1.229	1.39	0.87	0.76
3	PHB	4.48	0.990	0.90	0.81	0.56
4	FER	4.58	1.429	1.53	0.80	0.91
5	VAN	4.66	1.190	1.33	0.94	0.72
6	SIN	4.61	1.628	1.72	0.81	1.19
7	SYR	4.98	1.390	1.75	0.75	0.89
8	HPA	4.36	1.131	1.45	0.94	0.74
9	PRO	4.48	1.049	1.45	1.25	0.66
10	CAF	4.62	1.288	1.55	1.36	0.90
11	GAL	4.50	1.108	1.69	1.63	0.86
12	CLG	2.66	2.416	2.61	2.08	2.49

As shown in several examples in Fig. 3, phenolic acids are weakly retained on the un-modified Silica hydride and Diamond hydride columns in highly aqueous mobile phases, which are not suitable for separations in the reversed-phase mode. The hydrosilated silica modified with non-polar ligands has more hydrophobic surface and consequently significantly enhanced retention in the RP mode. Hence, the UDC cholesterol and C_{18} bidentate columns show significant dual reversed-phase/normal-phase retention behavior, in agreement with the earlier reports by Pesek and Matyska [25,49] and can be used for RP separations in buffered mobile phases containing less than 25% acetonitrile. The mixed retention mechanism should be interpreted so that the in the mobile phase with medium concentration of acetonitrile, where both the HILIC and the RP mechanisms play important role, the resulting retention is very low. In the highly aqueous retention range, the RP mechanism is predominant and the HILIC (NP) contributions can mostly be neglected, whereas in organic-rich mobile phases the HILIC mechanism largely predominates over the RP mechanism.

The retention factors, k, of all phenolic acids increase at decreasing concentration of the aqueous ammonium acetate buffer in the organic-rich HILIC mobile phases and increase in highly aqueous mobile phases (RP mode). The experimental data show characteristics U-shape plots of retention factors versus the concentration of aqueous buffer in the mobile phase, φ, on all four hydrosilated silica columns: silica hydride, C_{18} bidentate, UDC cholesterol and Diamond hydride. Some examples are shown in Fig. 3. The un-modified silica hydride and the diamond hydride columns show very low retention under RP conditions, as well as the C_{18} bidentate and UDC cholesterol columns in the intermediate mobile phase range from 25% to 85% acetonitrile, where the solvophobic and the polar interaction effects obviously largely compensate each other.

The four-parameter Eq. (2) fits better the experimental retention data than the three-parameter Eq. (4) over the broad composition range of the mobile phase, including the low φ HILIC range and the high φ RP range (full lines in Fig. 3). Table 3 lists the best-fit parameters a, m_{HILIC}, m_{RP} and b of Eq. (2). High values of the multiple corelation coefficients, R^2, and relatively low standard deviations of the parameters demonstrate good validity of Eq. (2) to describe the dual HILIC/RP retention model for phenolic acids on hydrosilated silica columns.

Figs. 4 and 5 show HILIC separations of eight phenolic acids on un-modified Silica hydride, Diamond hydride, UDC cholesterol and bidentate C_{18} columns in 95% acetonitrile containing 10 mmol/L ammonium acetate. For better comparison sake, the elution volumes, V_R, are normalized with respect to the column volume, V_C. The elution order is similar, but the retention decreases

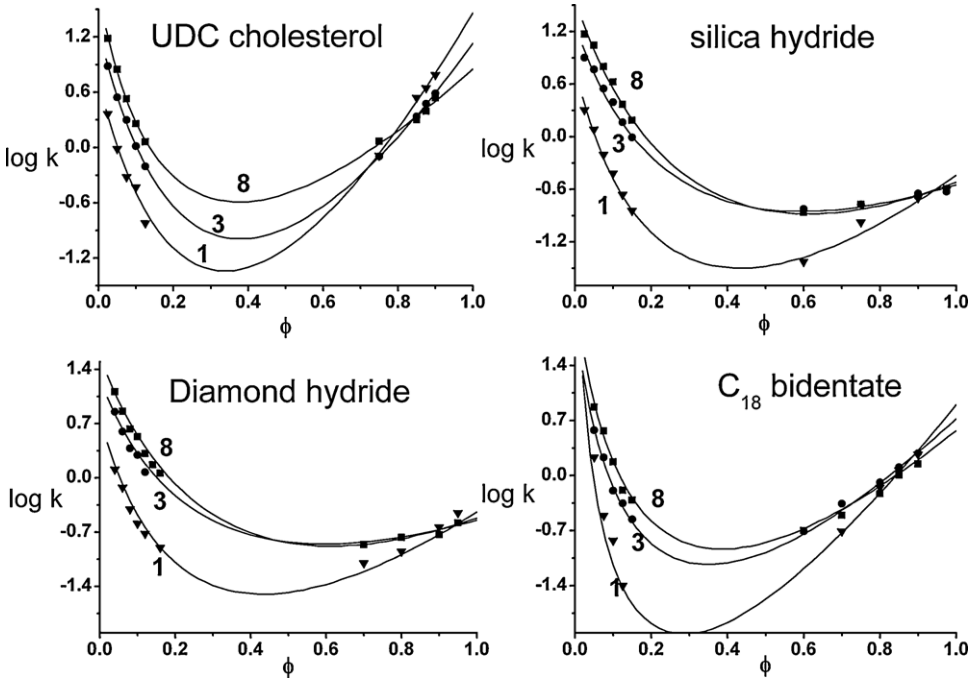

Fig. 3. Effects of the volume fraction of aqueous buffer (10 mM ammonium acetate, pH 3.26), Φ (10^{-2} vol.%), on the retention factors, k, of phenolic acids. Temperature 40 °C; flow rate, F_m = 0.5 mL min^{-1}; sample volume 10 μL. The numbers of acids are as in Fig. 1. Points: experimental data, lines: best-fit plots of Eq. (2).

Table 3
Best-fit parameters a, m_{RP}, m_{HILIC} and b of Eq. (2) ± standard deviations on hydrosilated columns. R^2: multiple correlation coefficients. The numbers of phenolic acids are as in Fig. 2.

Compound	A – Silica hydride					B – C_{18} bidentate				
	a	m_{RP}	m_{HILIC}	b	R^2	a	m_{RP}	m_{HILIC}	b	R^2
1	0.80 ± 0.02	6.16 ± 0.22	8.17 ± 0.21	7.06 ± 0.38	99.24	0.72 ± 0.02	13.12 ± 0.91	18.35 ± 0.53	3.72 ± 0.08	99.24
2	0.97 ± 0.05	5.22 ± 0.11	8.12 ± 0.41	6.32 ± 0.07	99.50	1.01 ± 0.04	10.15 ± 0.68	19.27 ± 0.95	3.14 ± 0.06	99.22
3	1.29 ± 0.29	3.89 ± 0.10	6.91 ± 0.05	5.74 ± 0.27	99.06	1.28 ± 0.08	11.14 ± 0.79	16.01 ± 0.71	4.09 ± 0.24	99.81
4	1.34 ± 0.01	5.21 ± 0.03	9.50 ± 0.32	4.41 ± 0.16	98.98	1.26 ± 0.03	23.86 ± 0.97	57.72 ± 2.11	1.51 ± 0.02	99.94
5	1.36 ± 0.07	4.05 ± 0.17	7.17 ± 0.12	5.62 ± 0.24	99.11	1.30 ± 0.01	13.31 ± 0.43	22.29 ± 0.11	2.96 ± 0.21	99.90
6	1.40 ± 0.02	5.10 ± 0.12	9.31 ± 0.23	4.51 ± 0.09	98.78	1.30 ± 0.05	29.86 ± 1.32	88.07 ± 1.66	11.35 ± 0.47	99.96
7	1.50 ± 0.07	4.19 ± 0.07	7.56 ± 0.31	5.52 ± 0.17	99.40	1.47 ± 0.07	18.40 ± 0.33	37.26 ± 1.78	2.11 ± 0.13	99.92
8	1.56 ± 0.01	5.39 ± 0.19	10.77 ± 0.09	3.94 ± 0.02	99.27	1.71 ± 0.02	6.43 ± 0.56	7.10 ± 0.40	9.61 ± 0.53	99.43
Compound	C – UDC cholesterol					D – Diamond hydride				
	a	m_{RP}	m_{HILIC}	b	R^2	a	m_{RP}	m_{HILIC}	b	R^2
1	3.46 ± 0.08	8.33 ± 0.26	5.73 ± 0.16	78.42 ± 2.11	98.83	11.11 ± 0.06	6.35 ± 0.06	18.91 ± 0.09	0.74 ± 0.04	94.21
2	1.14 ± 0.01	6.94 ± 0.33	6.15 ± 0.36	11.42 ± 0.50	97.55	8.74 ± 0.08	−6.70 ± 0.08	2.19 ± 0.11	14.5 ± 0.11	99.01
3	2.05 ± 0.07	7.10 ± 0.40	6.68 ± 0.21	17.32 ± 0.25	98.43	9.15 ± 0.15	−7.34 ± 0.15	2.23 ± 0.16	9.18 ± 0.03	98.15
4	2.12 ± 0.05	7.29 ± 0.19	6.24 ± 0.10	18.82 ± 0.98	98.31	1.21 ± 0.03	6.34 ± 0.15	10.21 ± 0.52	4.12 ± 0.02	99.12
5	2.09 ± 0.03	6.30 ± 0.25	5.75 ± 0.18	20.00 ± 0.71	98.66	1.45 ± 0.05	5.12 ± 0.25	7.28 ± 0.41	5.51 ± 0.17	99.29
6	1.97 ± 0.01	8.33 ± 0.37	7.82 ± 0.43	12.56 ± 0.18	98.14	1.51 ± 0.02	5.01 ± 0.07	8.91 ± 0.31	4.01 ± 0.12	98.17
7	2.01 ± 0.08	7.62 ± 0.20	7.70 ± 0.13	12.40 ± 0.35	98.56	1.65 ± 0.01	3.99 ± 0.09	7.21 ± 0.28	6.06 ± 0.35	97.28
8	2.31 ± 0.07	6.63 ± 0.08	6.87 ± 0.23	15.59 ± 0.61	99.04	1.78 ± 0.08	4.79 ± 0.26	10.21 ± 0.51	3.21 ± 0.18	99.53

with decreasing polarity of the stationary phase surface: Silica hydride > Diamond hydride > cholesterol > C_{18}. The Silica hydride and Diamond hydride columns provide better HILIC separation of the ferulic (4) and vanillic (5) acids than the columns with hydrophobic surface modification at 40 °C in buffered mobile phase containing 5% aqueous component in acetonitrile. The Diamond hydride column (Fig. 5, top) provides faster isocratic separation of 8 phenolic acids than the Silica hydride column (Fig. 4, top); the time of separation can be further decreased using gradient with increasing concentration of the aqueous component, from 2% to 10% in 10 min (Fig. 5, bottom).

The parameters m', s', a' and b' of Eq. (5), which characterize the hydrosilated silica based column selectivity contributions to the retention in the HILIC and in the RP mode are listed in Table 4. The experimental retention behavior can be explained on the basis of the data in this table. Negative values of m' and of a' in the HILIC range mean that the retention decreases for acids with larger size and with stronger hydrogen bonding acceptor properties, whereas positive s' and b' parameters indicate increasing retention for more polar compounds and (or) stronger hydrogen-bonding donor properties.

The experimental retention behavior can be explained on the basis of the data in these tables. The results are discussed in the text as follows: Negative values of m' and of a' in the HILIC range mean that the retention decreases for acids with larger size and with stronger hydrogen bonding acceptor properties, whereas positive s' and b' parameters indicate increasing retention for more polar compounds and (or) stronger hydrogen-bonding donor properties. The results illustrate predominating effects of hydrogen-bonding interactions in HILIC chromatography of phenolic acids. (Possible ion-exchange interactions with phenolic acids are included in the term b').

For example, salicylic acid shows intramolecular hydrogen bonding which affects lower values of dipole–dipole parameter pi and hydrogen bonding acceptor parameter beta in comparison to the parameters for sinapic acid (Table 2). As the HILIC column

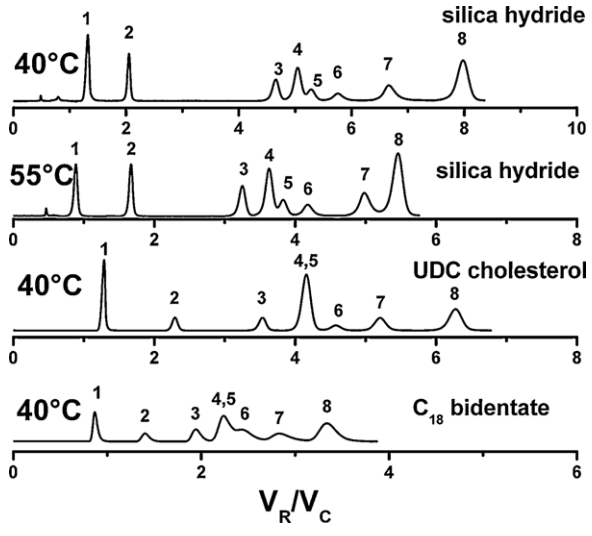

Fig. 4. Separation of phenolic acids in the HILIC mode. Mobile phase: 10 mM NH_4AC in 5:95% water:acetonitrile, pH 3.26, F_m = 0.5 mL min^{-1}, λ = 275 nm, sample volume: 10 μL. V_R: retention volume, and V_C: volume of the empty column.

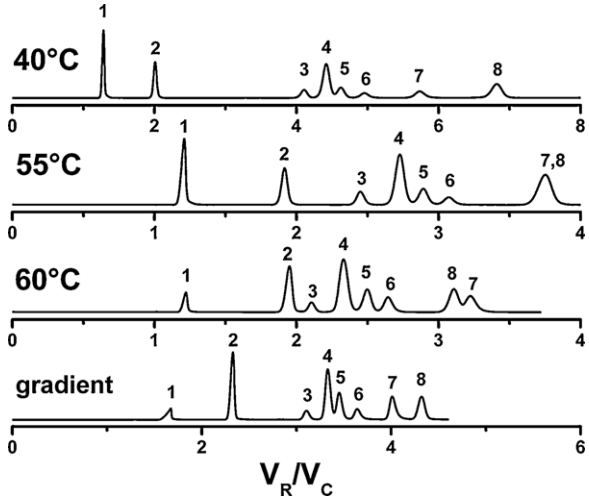

Fig. 5. HILIC separations of phenolic acids on a Diamond hydride column. Isocratic conditions: mobile phase: 10 mM NH_4AC in 5:95% water:acetonitrile, pH 3.26, gradient conditions: 2–10% B in 10 min. (A) 10 mM NH_4Ac in ACN, (B) 10 mM NH_4Ac in water (pH 3.26, adjusted with HCOOH), 40 °C. F_m = 0.5 mL min^{-1}, λ = 275 nm, sample volume 10 μL. V_R: retention volume, and V_C: volume of the empty column.

Table 4
The parameters log k', m', s', a' and b' (Eq. (5)) of phenolic acids.

Column	log k_0'	m'	s'	a'	b'	R^2
HILIC φ(ACN) = 0.95						
Silica hydride	2.411	−3.453	0.615	−0.710	3.057	0.9316
Diamond hydride	2.218	−3.481	0.672	−0.529	3.156	0.9759
UDC cholesterol	1.872	−3.005	0.795	−0.629	2.456	0.9649
C_{18} bidentate	1.889	−2.927	0.582	−0.619	2.614	0.9850
RP φ(ACN) = 0.15						
UDC cholesterol	−1.078	2.530	−0.088	−0.555	−1.500	0.9885
C18 bidentate	−0.919	2.788	−0.242	−0.243	−1.712	0.9983

parameters s' and b' are positive, higher pi and beta values result in stronger retention of sinapic acid. The b' (hydrogen bonding) parameters are larger (characterizing more important contribution to the retention of phenolic acids) than the dipole interaction parameters s' and as b' are higher for the silica hydride column, this column shows larger differences in hydrogen-bonding and consequently in overall separation selectivity between the sinapic and salicylic acids than the Cholesterol column.

3.2. Influence of temperature on the HILIC separation process

Temperature has very important effect on the separation process. We investigated the retention of phenolic acids in the temperature range from 35 °C up to the column stability limits (60 °C for the Diamond hydride column, 80 °C for the C_{18} bidentate column and 100 °C for the UDC cholesterol and Silica hydride columns) in buffered ACN–water mobile phases. The retention data at lower temperature settings may be less accurate when using an air circulated thermostat without external cooling facility and therefore were not included. The retention and – to some extent – the peak widths decrease at higher temperatures and the separation improves when increasing the temperature from 40 °C to 55 °C on the Silica hydride column (Fig. 4) or to 60 °C on the Diamond hydride column (Fig. 5), but also the selectivity of separation may change significantly at increasing temperature. At the high temperature column stability limits (100 °C for the UDC cholesterol column, 80 °C for the C_{18} bidentate column and 100 °C for the silica hydride column) most phenolic acids were too weakly retained and could not be separated even at very low concentration of aqueous component (2%) in the mobile phase.

Tables 5 and 6 show the best-fit regression parameters of Eq. (3), i.e., the intercepts, A_i, the slopes, B_i, and the correlation coefficients, R^2 of the experimental log k versus $1/T$ data plots, measured under HILIC conditions in acetonitrile containing 5% 10 mmol/L ammonium acetate buffer. High correlation coefficients of the experimental data plots in Fig. 6 demonstrate the validity of Eq. (3) for all the phenolic acids on the columns tested under HILIC conditions, indicating that a single retention mechanism controls the retention over a broad temperature range. For all columns, the plots are significantly less steep for weakly retained salicylic and coumaric acids.

To explain this behavior, we calculated the thermodynamic data for the transfer of each phenolic acid from the mobile phase to the stationary phase: the standard partial molar entropy, ΔS^0, from the intercepts, A_i, and the standard partial molar enthalpy, ΔH^0, from the slopes, B_i, of Eq. (3), using the convention for the definition of the column phase ratio according to Eq. (4). The role of the enthalpic and entropic contributions at a specific experimental temperature can be estimated by comparing the numerical values of B_i/T and A_i, respectively (Tables 5 and 6). Of course, the enthalpic contributions, B_i/T, decrease at higher temperatures and are significantly higher than the entropic contributions at low (40 °C) as well as at high (90 °C) temperatures on the Silica hydride and Diamond hydride columns (Table 5). However, the entropic contributions, A_i are more significant with the modified UDC cholesterol and C_{18} bidentate columns in the HILIC mode and at high temperatures may be similar to the enthalpic contributions, or even slightly higher for weakly retained salicylic (1) and coumaric (2) acids (Table 5).

Decreasing retention and shorter analysis times at increasing temperature may be connected with changed elution order such as of ferulic (4) and vanillic (5) acids on the Diamond hydride column, where their separation significantly improves at 55 °C, or 60 °C, unlike on the Silica hydride column. 4-hydroxyphenylacetic acid (8) shows the highest enthalpic contribution to the retention on the Diamond hydride column and its retention decreases far more significantly when increasing the temperature in comparison

Table 5
Best-fit parameters A_i and B_i of Eq. (3) ±standard deviations. HILIC conditions: mobile phase 10 mM NH_4AC in 5:95% water:acetonitrile + 45 μL/L HCOOH, F_m = 0.5 mL min^{-1}, silica hydride column temperature interval 35–100 °C, Diamond hydride column temperature interval 35–60 °C, λ = 275 nm, and injection volume 10 μL. R^2: correlation coefficients. The numbers of phenolic acids are as in Fig. 2.

		A_i	B_i	R^2	$-\Delta H^0$ (kJ/mol)	ΔS^0 (J/mol K)	B_i/T (40 °C)	B_i/T (90 °C)
Silica hydride								
1	SAL	−0.92 ± 0.03	496.6 ± 25.4	0.9521	−4.13	−9.07	1.59	1.37
2	COU	−1.11 ± 0.04	762.1 ± 11.2	0.9632	−6.34	−10.65	2.43	2.10
3	PHB	−10.66 ± 0.25	4057.9 ± 130.7	0.9892	−33.74	−90.05	12.96	11.17
4	FER	−10.18 ± 0.12	3938.4 ± 64.1	0.9934	−32.74	−86.06	12.58	10.85
5	VAN	−10.09 ± 0.51	3928.1 ± 19.5	0.9917	−32.66	−85.31	12.54	10.82
6	SIN	−10.38 ± 0.21	4049.4 ± 22.7	0.9914	−33.67	−87.72	12.93	11.15
7	SYR	−9.66 ± 0.23	3877.1 ± 76.7	0.9894	−32.23	−81.74	12.38	10.68
8	HPA	−11.89 ± 0.49	4627.4 ± 152.1	0.9919	−38.47	−100.28	14.78	12.74
Diamond hydride								
1	SAL	−0.83 ± 0.02	567.3 ± 9.1	0.9952	−4.72	−2.63	1.81	1.56
2	COU	0.06 ± 0.01	472.1 ± 18.5	0.9661	−3.93	4.77	1.51	1.30
3	PHB	−10.38 ± 0.77	3997.2 ± 102.1	0.9988	−33.23	−82.02	12.76	11.01
4	FER	−9.52 ± 0.42	3752.3 ± 51.6	0.9988	−31.20	−74.87	11.98	10.33
5	VAN	−9.06 ± 0.32	3623.9 ± 147.2	0.9989	−30.13	−71.05	11.57	9.98
6	SIN	−9.16 ± 0.21	3680.8 ± 99.5	0.9986	−30.60	−71.88	11.75	10.14
7	SYR	−8.12 ± 0.33	3405.8 ± 44.6	0.9946	−28.32	−63.23	10.88	9.38
8	HPA	−11.22 ± 0.35	4430.3 ± 87.5	0.9991	−36.83	−89.01	14.15	12.20

Table 6
Best-fit parameters A_i and B_i of Eq. (3) ± standard deviations and correlation coefficients, R^2. HILIC conditions: mobile phase composition – 10 mM NH_4AC in 5:95% water:acetonitrile + 45 μL/L HCOOH, F_m = 0.5 mL min^{-1}, UDC cholesterol column: temperature interval 35–100 °C, C_{18} bidentate column: temperature interval 35–80 °C, λ = 275 nm, and injection volume 10 μL. The numbers of phenolic acids are as in Fig. 2.

		A_i	B_i	R^2	$-\Delta H^0$ (kJ/mol)	ΔS^0 (J/mol K)	B_i/T (40 °C)	B_i/T (90 °C)
UDC cholesterol								
1	SAL	−5.80 ± 0.22	2006.4 ± 89.1	0.9903	−16.68	−30.05	6.41	5.52
2	COU	−6.43 ± 0.12	2459.9 ± 50.7	0.9907	−20.45	−34.29	7.86	6.77
3	PHB	−13.41 ± 0.52	4804.4 ± 65.9	0.9962	−39.94	−85.67	15.34	13.23
4	FER	−13.16 ± 0.23	4788.1 ± 28.8	0.9969	−39.81	−70.46	15.29	13.18
5	VAN	−13.03 ± 0.44	4747.3 ± 199.5	0.9968	−39.47	−76.19	15.16	13.07
6	SIN	−13.39 ± 0.35	4894.6 ± 50.4	0.9966	−40.69	−80.02	15.63	13.48
7	SYR	−12.78 ± 0.51	4754.5 ± 112.3	0.9963	−39.53	−76.52	15.18	13.09
8	HPA	−15.20 ± 0.43	5574.5 ± 78.6	0.9972	−46.35	−94.07	17.80	15.35
C_{18} bidentate								
1	SAL	−3.28 ± 0.17	1014.4 ± 20.1	0.9644	−8.43	−11.72	3.24	2.79
2	COU	−3.79 ± 0.11	1454.8 ± 31.5	0.9444	−12.10	−15.96	4.65	4.01
3	PHB	−9.97 ± 0.35	3525.8 ± 83.7	0.9981	−29.31	−67.34	11.26	9.71
4	FER	−8.14 ± 0.21	2986.4 ± 105.9	0.9915	−24.83	−52.12	9.54	8.22
5	VAN	−8.83 ± 0.41	3231.3 ± 122.6	0.9849	−26.87	−57.86	10.32	8.90
6	SIN	−9.29 ± 0.38	3407.1 ± 51.7	0.9836	−28.33	−61.68	10.88	9.38
7	SYR	−8.87 ± 0.14	3334.9 ± 41.8	0.9939	−27.73	−58.19	10.65	9.18
8	HPA	−10.98 ± 0.46	4053.2 ± 67.8	0.9900	−33.70	−75.73	12.94	11.16

to the other acids. This behavior dramatically affects the separation of syringic acid (7) and hydroxyphenylacetic acid (8). The two compounds are over-resolved at 40 °C, co-elute at 55 °C, and are partially separated, but in the reversed elution order, at 60 °C. On the other hand, increasing temperature does not change significantly the retention of salicylic (1) and coumaric (2) acids, which is controlled mainly by entropic effects. Obviously, the effects of temperature on the separation selectivity of phenolic acids on the diamond hydride column in the HILIC retention range are more important than the effect of decreasing concentration of acetonitrile during gradient elution (Fig. 5, bottom).

3.3. Reversed-phase separation of phenolic acids on hydrosilated columns

In the RP mode, the UDC cholesterol and C_{18} bidentate columns provide different elution order of the phenolic acids studied (but not completely reversed) and improved separation in comparison to the HILIC mode (Fig. 7). The separation selectivity on the two columns is very similar and largely independent of temperature; however the elution is much faster at the cost of impaired resolution at 80 °C or 100 °C with respect to the separation at 40 °C. At the same conditions (mobile phase, temperature), the phenolic acids are more retained and better separated on the UDC cholesterol than on the C_{18} bidentate column – 12 phenolic acids can be almost completely resolved in approximately 5 column volumes of the buffered mobile phase containing 15% acetonitrile at 40 °C. The protocatechuic, gallic and chlorogenic acids, Nos. 9, 11 and 12, which are too strongly retained in the HILIC mobile phase range, elute early under the RP conditions and are well separated from each other and from the other phenolic acids.

At high temperatures, lower retention times and impaired resolution of phenolic acids was observed in the RP mode, but the temperature affects much less the separation selectivity on the UDC

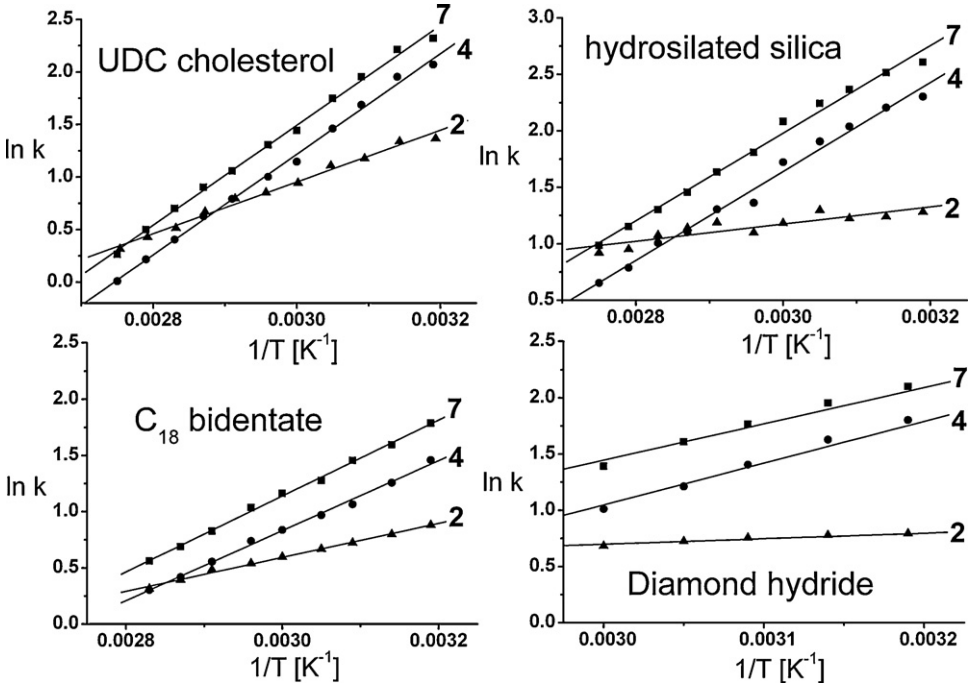

Fig. 6. Temperature effects on the retention factors, k, of phenolic acids in the HILIC mode. Mobile phase: 10 mM NH_4AC in 5:95% water:acetonitrile, pH 3.26, F_m = 0.5 mL min^{-1}, λ = 275 nm, sample volume 10 μL, T: thermodynamic temperature in K.

Table 7
Best-fit parameters A_i and B_i of Eq. (3) ± standard deviations and correlation coefficients, R^2. RP conditions: mobile phase composition – 10 mM NH_4AC in 85:15% water:acetonitrile (pH = 3.26), F_m = 0.5 mL min^{-1}, C_{18} bidentate column: temperature interval 35–80 °C,λ = 275 nm, injection volume 10 μL, and UDC cholesterol column: temperature interval 35–100 °C. The numbers of phenolic acids are as in Fig. 2.

		A_i	B_i	R^2	$-\Delta H^0$ (kJ/mol)	ΔS^0 (J/mol K)	B_i/T (40 °C)	B_i/T (90 °C)
C_{18} bidentate								
1	SAL	−4.42 ± 0.04	1686.9 ± 17.2	0.9934	−14.02	−37.63	5.39	4.65
2	COU	−7.61 ± 0.07	2766.8 ± 28.5	0.9978	−23.00	−64.16	8.84	7.62
3	PHB	−4.09 ± 0.21	1527.6 ± 68.7	0.9711	−12.70	−34.89	4.88	4.21
4	FER	−5.33 ± 0.02	2274.7 ± 19.8	0.9983	−18.91	−45.20	7.26	6.26
5	VAN	−4.09 ± 0.06	1610.3 ± 27.1	0.9943	−13.39	−34.89	5.14	4.43
6	SIN	−4.88 ± 0.03	2156.7 ± 14.8	0.9982	−17.93	−41.46	6.89	5.94
7	SYR	−4.42 ± 0.08	1812.3 ± 21.6	0.9966	−15.07	−37.63	5.79	4.99
8	HPA	−4.73 ± 0.02	1718.0 ± 19.5	0.9938	−14.28	−40.21	5.49	4.73
9	PRO	−5.20 ± 0.17	1705.2 ± 36.4	0.9887	−14.18	−44.12	5.45	4.70
10	CAF	−3.87 ± 0.12	1565.1 ± 60.9	0.9851	−13.01	−33.06	5.00	4.31
11	GAL	−6.22 ± 0.28	1854.1 ± 88.3	0.9775	−15.41	−52.60	5.92	5.11
12	CLG	−5.33 ± 0.16	1772.9 ± 38.6	0.9845	−14.74	−45.20	5.66	4.88
UDC cholesterol								
1	SAL	−5.75 ± 0.09	2257.6 ± 17.5	0.9920	−18.77	−48.69	7.21	6.22
2	COU	−5.80 ± 0.03	2374.7 ± 12.6	0.9974	−19.74	−49.11	7.58	6.54
3	PHB	−5.13 ± 0.12	1899.9 ± 32.1	0.9859	−15.80	−43.54	6.07	5.23
4	FER	−5.15 ± 0.05	2257.6 ± 15.8	0.9983	−18.77	−43.70	7.21	6.22
5	VAN	−4.27 ± 0.06	1703.7 ± 24.1	0.9904	−14.16	−36.39	5.44	4.69
6	SIN	−4.92 ± 0.02	2227.2 ± 11.1	0.9983	−18.52	−41.79	7.11	6.13
7	SYR	−3.15 ± 0.14	1394.3 ± 44.9	0.9753	−11.59	−27.08	4.45	3.84
8	HPA	−4.41 ± 0.09	1613.8 ± 26.5	0.9883	−13.42	−37.55	5.15	4.44
9	PRO	−4.62 ± 0.21	1593.5 ± 36.2	0.9772	−13.25	−39.30	5.09	4.39
10	CAF	−5.60 ± 0.05	2138.3 ± 21.8	0.9923	−17.78	−47.45	6.83	5.89
11	GAL	−4.58 ± 0.18	1391.0 ± 41.5	0.9776	−11.56	−38.96	4.44	3.83
12	CLG	−5.17 ± 0.05	1797.6 ± 20.5	0.9904	−14.95	−43.87	5.74	4.95

cholesterol and C_{18} bidentate columns than in the HILIC mode. In agreement with Eq. (3), linear log k versus $1/T$ plots were observed (Fig. 8), however less steep than in the HILIC mode (Fig. 6), due to lower enthalpy of retention, ΔH^0, in the RP mode – Table 7 – (except for the salicylic (1) and the coumaric (2) acids), lower in comparison to the corresponding enthalpies in the HILIC mode (Table 6). Also the entropy of retention, ΔS^0, is less negative in the RP than in the HILIC mode (again except for salicylic (1), and coumaric (2) acids, which are weakly retained under HILIC conditions). Table 7 shows the results of the regression of the experimental log k versus $1/T$ data set in the RP mode: the best-fit value of the intercepts, A_i, the slopes, B_i, of Eq. (3), the correlation coefficients, R^2, the standard partial molar entropy, ΔS^0, and the standard partial molar enthalpy, ΔH^0, of the transfer of the solute from the mobile phase to the stationary phase. For most phenolic acids, the enthalpic contributions to the retention, B_i/T, are more significant than the entropic contributions, A_i, on the UDC cholesterol and C_{18} bidentate columns, both at low (40 °C) and high (90 °C) temperatures (Table 7). However, the phenolic acids with 2 (protocatechuic, No. 9, chlorogenic, No. 12) or 3 (gallic, No. 11) phenolic –OH groups (and 4-hydroxyphenylacetic acid, No. 8), which are very strongly

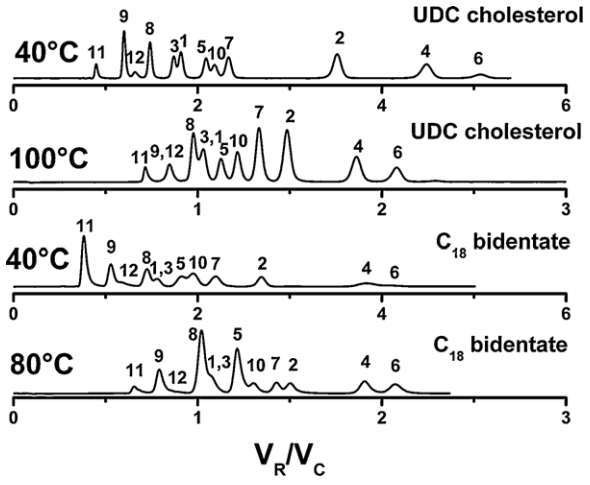

Fig. 7. Separations of phenolic acids in the reversed-phase mode on the C_{18} bidentate and UDC cholesterol columns. Mobile phase: 10 mM NH_4AC in 85:15% water:acetonitrile, pH 3.26, F_m = 0.5 mL min^{-1}, λ = 275 nm, sample volume 10 μL. V_R: retention volume, and V_C: volume of the empty column.

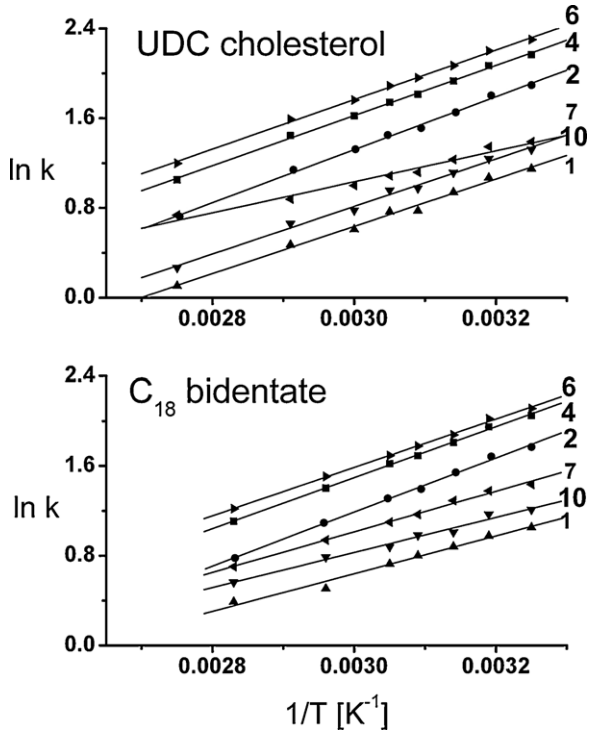

Fig. 8. Temperature effects on the retention factors, k, of phenolic acids in the RP mode. Mobile phase: 10 mM NH_4AC in 85:15% water:acetonitrile, pH 3.26, F_m = 0.5 mL min^{-1}, λ = 275 nm, sample volume 10 μL, T: thermodynamic temperature in K.

retained in the HILIC mode, elute early under the RP conditions and show entropic contributions to the retention comparable with (at 40 °C), or even higher than (at 90 °C) the enthalpic contributions.

4. Conclusions

Hydrosilated silica (type C) columns show dual retention mechanism in aqueous–organic mobile phases – HILIC in highly organic mobile phases and RP at high concentrations of water. The effects of the composition of buffered aqueous acetonitrile mobile phases on the retention strongly depend on the type of hydrosilated silica stationary phase and can be accurately described using a relatively simple four-parameter equation, Eq. (2), over the full mobile phase composition range including both the HILIC and the RP retention areas.

The unmodified Silica hydride and the Diamond hydride columns can be used only for HILIC separations of phenolic acids, while the less polar hydrosilated silica columns modified with C_{18} and cholesterol ligands provide useful separation in both the HILIC and the RP mode, with (almost) reversed order of elution and essentially changed separation selectivity. Whereas phenolic acids containing two or more phenolic –OH groups are too strongly retained in the HILIC mode and provide broad and unsymmetrical peaks, they can be readily separated under RP conditions on the C_{18} bidentate column and especially on the UDC cholesterol column, which provides almost complete separation of 12 phenolic acids in approximately five column volumes. An obvious advantage of the RP mode is lower consumption of acetonitrile in the mobile phase.

The Silica hydride, C_{18} bidentate and UDC cholesterol columns show improved thermal stability (up to 80–100 °C) and lower polarity with respect to most conventional columns based on the silica type B. Stronger adsorption of methanol on the silica hydride material in comparison to the classical silica type B reported in a recent work [50] is in agreement with lower polarity of the Silica hydride. In aqueous–organic mobile phases, water is more polar than either methanol or acetonitrile and hence is more strongly adsorbed on the Si–OH silanol groups, which are thus desactivated and less accessible for methanol than less polar Si–H groups, which prefer less polar organic solvents to water. The retention times and bandwidths decrease and the overall separation improve at increasing temperature in the HILIC mode, especially on the Silica Hydride and Diamond hydride columns. In the RP mode, the separation selectivity of some phenolic acids impairs at higher temperature, which may overweigh the advantages of shorter analysis time and improved peak widths. The $\log k$ of phenolic acids show linear change with $1/T$, in agreement with the van't Hoff model, both in the RP and in the HILIC mode. On the Silica hydride and Diamond hydride columns, the enthalpic contributions to the retention are significantly higher than the entropic contributions in the HILIC mode, but the differences are significantly lower on the C_{18} bidentate and UDC cholesterol columns, where they are almost comparable at high temperatures and the entropic effects even may predominate over the enthalpic ones for weakly retained phenolic acids with two or more phenolic –OH groups in the RP mode, or with salicylic and p-coumaric acids in the HILIC mode. From among all the columns tested, the UDC cholesterol column fits the best for the dual mode HILIC and RP separations of phenolic acids, except for the resolution of ferulic and vanillic acids, which is better on the Diamond hydride column. This may be due to the differences in selectivity caused by additional interactions of the phenolic acid –OH and –COOH groups with the bonded cholesterol phase. High thermal stability up to 100 °C is undoubtedly important advantage of this silica type C column in comparison to less stable columns based on the silica B type.

The present results demonstrate the importance of temperature as a complementary tool to the mobile phase composition for the control and optimization of separation on the un-modified and modified hydrosilated silica (type C) columns, showing dual HILIC/RP retention mechanism.

Acknowledgments

This work was supported by the by the Grant Agency of the Czech Republic, project No. 203/07/0641 and by the Ministry of Education, Youth and Sports of the Czech Republic, project No. 253100002. We are grateful to Prof. Joseph Pesek (San Jose University, CA) for the gift of the Silica hydride column.

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