Evaluation of an amide-based stationary phase for supercritical fluid chromatography

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Abbreviations: SFC, Supercritical Fluid Chromatography; SF, Supercritical Fluid; LSER, Linear Solvation Energy Relationship.

Keywords: Linear solvation energy relationship / Retention prediction /Stationary phases / Supercritical fluid chromatography

Abstract

A relatively new stationary phase containing a polar group embedded in a hydrophobic backbone (i.e., ACE®C18-amide) was evaluated for use in supercritical fluid chromatography. The amide-based column was compared with columns packed with bare silica, C18 silica, and a terminal-amide silica phase. The system was held at supercritical pressure and temperature with a mobile phase composition of carbon dioxide and methanol as co-solvent. The linear solvation energy relationship model was used to evaluate the behavior of these stationary phases, relating the retention factor of selected probes to specific chromatographic interactions. A five-component test mixture, consisting of a group of drug-like molecules was separated isocratically. The results show that the C18-amide stationary phase provided a combination of interactions contributing to the retention of the probe compounds. The hydrophobic interactions are favorable; however, the electron donating ability of the embedded amide group shows a large positive interaction. Under the chromatographic conditions used, the C18-amide column was able to provide baseline resolution of all the drug-like probe compounds in a text mixture, while the other columns tested did not.

1 Introduction

Supercritical fluid chromatography (SFC) continues to proliferate as an environmentally friendly separation technique, particularly in a format similar to that of packed column liquid chromatography (LC). A historical perspective on the development of SFC, the current state of the art, and how the technique has gained popularity are readily available in the current literature [1–7]. Today, SFC is mostly practiced using CO_2 as the mobile phase with methanol, or other alcohols [8, 9], as co-solvent modifiers that can be adjusted during the separation via gradient elution, if necessary, to increase mobile phase elution strength [10].

It is important to note that as it is practiced, addition of additives to the CO_2 mobile phase causes an increase in the critical parameters. However, the mobile phase does not necessarily have to be at its critical state and excellent separations can be obtained at subcritical conditions [11]. There may even be situations in which a gradient involving the addition of a modifier to the CO_2 mobile phase changes the conditions from supercritical to near critical conditions during the separation process. This is possible because of the continuum of properties when moving from the sub- to supercritical region [12–14].

The retention and separation of compounds in SFC depend on a combination of factors that involve the characteristics of the mobile and the stationary phases inside the chromatographic column. The characteristics of the mobile phase in SFC can provide for tunable selectivity, although the stationary phase also affects selectivity in SFC. Most of the stationary phases used in SFC are extensions of those used in HPLC, although stationary phases designed for achiral SFC have been explored [15–18].

The linear solvation energy relationship (LSER) model using Abraham [19–23] descriptors has acquired favorable acceptance in SFC to characterize column selectivity [11, 24–28]. This model has been used to rationalize the intermolecular processes that lead to the separation of solutes. In the LSER model, the chromatographic retention factor (k) of selected analytes is related to specific interactions according to the following relationship:

$$\log k = c + e\mathrm{E} + s\mathrm{S} + a\mathrm{A} + b\mathrm{B} + v\mathrm{V} \quad (1)$$

where c is the model intercept; E, S, A, B, and V are the solute descriptors and e, s, a, b, and v are coefficients attributed to the system. In the case of the solute descriptors: E is the excess molar refraction, S is the solute dipolarity/polarizability, A is the solute overall acidity, B is the overall basicity of the solute, and V is the McGowan characteristic volume. V is an approximation for the molecular volume in units of $cm^3 \cdot mol^{-1} \cdot 100^{-1}$. It is calculated by adding the atomic volumes, then subtracting 6.56 $cm^3 \cdot mol^{-1}$ for each bond of any type. E is the molar refraction of the compound minus the molar refraction of an alkane with the same V, in units of $cm^3 \cdot mol^{-1} \cdot 10^{-1}$ [29]. Solute descriptors A, B, and S are obtained by mathematical procedures from physicochemical measurements, such as partition coefficient (P) values in a number of water–solvent systems. These solute descriptors are obtain by TripleX, Solver, Descfit, or Regression programs [21]. These descriptors are readily available in the literature [11, 27, 30]. The system constants relate to the different chromatographic interactions, extracted by multiple linear regression analysis, for a particular chromatographic system and are defined as follows [11, 27, 30]. The e parameter represents the interactions through nonbonding n- and p-electrons; the s term measures the system ability to interact via dipole–dipole interactions; the a term is a measure of the ability to donate a lone pair of electrons or accept H-bond (system

100 basicity); *b* measures the ability to accept a lone pair of electrons or donate H-bond
101 (system acidity); *v* measures the hydrophobic interaction between the mobile phase
102 and the stationary phase; and *c* is the model intercept term. We note that the model is
103 in ongoing refinement evidenced by recent modifications to the equation in order to
104 account for ionic interactions [31, 32].

105 A large inventory of chromatographic columns has been characterized via
106 LSER [11, 24, 26, 27] under SFC conditions. However, new stationary phases are
107 introduced and their characterization and comparison with existing ones is
108 meritorious. Herein, we report on the characterization of a relatively new amide-
109 embedded stationary phase (i.e., ACE® C18-amide) as adsorbent for SFC. Using the
110 LSER model, we evaluated and compared the C18-amide column with three other
111 columns (a bare silica, a C18, and a terminal-amide column) under SFC conditions. A
112 group of five small drug-like molecules in a test mixture was used to examine the
113 separation ability of all the columns studied under a given set of isocratic conditions.
114

115 ## 2 Material and Methods

116 ### 2.1 Chemicals

117 The test solutes used in this study were obtained from various of suppliers.
118 Toluene, propylbenzene, butylbenzene, biphenyl, phenol, benzoic acid, aniline, N, N-
119 dimethylaniline, caffeine, o-cresol, p-cresol, m-cresol, phloroglucinol, bromobenzene,
120 chlorobenzene, nitrobenzene, anisole, naphthoic acid, acetophenone, 2,4-
121 dimethylphenol, 2,6-dimethylphenol, p-nitrophenol, o-nitrophenol, m-nitrophenol,
122 uracil, naproxen, ibuprofen, nifedipine, and bupropion were acquired from Sigma-
123 Aldrich, Inc. Benzaldehyde, naphthalene, and benzyl alcohol were purchased from
124 Thermo Fisher Scientific, Inc. All chemicals were used as received without any

125 further purification. Individual samples were prepared in methanol (Thermo Fisher
126 Scientific, Inc.) as solvent at a concentration of ranging from 10 to 2 mM; the mixture
127 containing the various components was prepared in methanol at a concentration of 2
128 mM each. Samples were filtered through a 0.45 μm membrane filter prior to injection
129 into the chromatograph. HPLC grade methanol from Thermo Fisher Scientific, Inc.
130 was used as the mobile phase modifier. Food grade carbon dioxide was purchased by
131 PRAXAIR, Inc. and used as the mobile phase.
132

133 **2.2 Chromatographic system and conditions**

134 The chromatographic system used consisted of an 1200 Series Agilent
135 Technologies HPLC equipped with a Fusion™ A5 SFC conversion module (Aurora
136 SFC System, Inc.). The HPLC system was composed of a binary pump, solvent
137 cabinet, well plate auto sampler, thermostated column compartment (TCC), Model
138 1200C diode array detector, and a degasser. The Agilent ChemStation software
139 controlled the system. Detection of the solutes was accomplished at 220 nm and/or
140 254 nm. The columns used in the study were the 3 μm ACE® C18-amide (3.0 mm i.d.
141 × 150 mm length) from MAC-MOD (Chadds Ford, PA), the 1.7 μm ACQUITY
142 UPLC BEH amide (2.1 mm i.d. × 150 mm length) from Waters (Milford, MA), the 5
143 μm YMC Pack Pro C18, (4.6 mm i.d. × 250 mm length) from YMC America,
144 (Allentown, PA), and the 5 μm Zorbax Sil (4.6 mm × 250 mm length) from Agilent
145 Technologies (Santa Clara, CA).

146 The mobile phase consisted of CO_2-methanol 95:5 (v/v). The temperature was
147 set at 80 °C and the outlet pressure was maintained at 175 bar. For the pressure
148 difference to be maintained at 50 bar for all the stationary phases, the flow rate was
149 3.0 mL·min^{-1} for columns YMC Pack Pro C18, and Zorbax Sil, 1.5 mL·min^{-1} for the

ACE® C18-amide, and 0.6 mL·min^{-1} for the ACQUITY UPLC BEH amide. It should be noted that even do the same pressure drop was maintained to obtain comparable retention, the reduced linear velocities for each column would be different. The injection volume was 1 μL for all tests unless indicated otherwise. The multilinear regression model and the statistical analysis of variance (ANOVA) were performed using OriginPro (OriginLab Corp., Northampton, MA).

3 Results and discussion

3.1 Fitting the model

The amide embedded C18 column was compared with three different columns, all silica based. The columns had different polarities: a bare silica column (high polarity), a C18 column (very low polarity), and a terminal-amide on a short linker chain. The LSER model was constructed using 24, 22, 22, and 21 different solutes varying in polarity for the C18, silica, terminal-amide, and C18-amide, respectively. The solutes and the descriptors used in the LSER model are presented in Table 1, which are readily available in the published literature [11, 27]. Three injections were performed for each solute and the average retention factor was used to construct the LSER model from which the system constants were extracted. The statistics related to the overall fit of the LSER model are the overall correlation coefficient (R), the standard error (SE), and the Fisher F-statistics test (ratio of the mean squares from the regression) [22, 33]. Outliers were detected for each stationary phase based on analysis of residuals (values of residuals rescaled by the standard error beyond -2.5 and 2.5 were considered outliers) [34], using a standard software package OriginPro; these were different for each stationary phase and eliminated from the set of solutes considered in the multilinear regression analysis.

175

176 **3.2 System constants**

177 The system constants extracted from multiregression analysis, their values,
178 and corresponding statistics are presented on Table 2. The model fits the data
179 reasonably well for all columns, with a strength of the linear association determined
180 by the correlation coefficient (R) with R-values ranging from 0.865 to 0.996, and SE
181 fluctuating from 0.058 to 0.187. The fit for each column can improve by increasing
182 the number of solutes considered in the regression; however, only five to six solutes
183 per descriptor are necessary to provide the information required to gain predictive
184 insight from the model on the system [35]. The Fisher test results, performed at 95%
185 confidence level, showed that there is a strong relationship between the dependent
186 variable (i.e., log *k*) and the independent variables (i.e., E, S, A, B, and V), meaning
187 that the linear regression model is a good fit for the data of all columns. We note that
188 the log *k* data obtained from the C18-amide column provided a better fit to the LSER
189 model than the other columns; plots of residuals clearly show this (see Figure S1 in
190 supplemental information).

191 Four compounds, not used to fit the model, that had a broad representation of
192 the values of the descriptors being considered in the model were used for the
193 assessment of the model prediction ability (i.e., 2,4-dimethylphenol, anisole, o-
194 nitrophenol, and aniline). Figure 1 shows a scatter plot of the predicted log *k* vs. the
195 observed log *k* for these compounds on the four stationary phases and its
196 corresponding residual plot; a linear fit of the data is also shown (R = 0.983.)
197 ANOVA analysis at a 95% confidence level indicated that there was no statistical
198 difference between the predicted and observed log *k* values.

Aside from the good fit, the coefficient values must make chemical sense. The magnitude of the descriptor represents the difference in the stationary and mobile phase interaction abilities and to which extent they dictate the overall property. The sign indicates which phase has greater interaction ability as represented by the particular descriptor. A positive sign reflects a greater interaction in the stationary phase, whereas a negative sign indicates a greater interaction in the mobile phase. A coefficient that is small or zero does not necessarily indicate that the interaction is non-existent, but rather that the interactions are of similar magnitude in both phases.

Figure 2 depicts the coefficient values obtained from the LSER model for all four stationary phases. Three of the four phases studied (i.e., C18, silica, and terminal-amide) followed the typical trend reported in the literature indicating that polar and nonpolar phases have opposite behavior under SFC conditions. For example, in polar phases the *v*-term is negative and the *s*, *a*, and *b*-coefficients are positive [30]. For non-polar phases the *v*-coefficient is positive, and *s*, *a*, and *b* are negative [36]. This makes chemical sense because non-polar analytes have less retention (negative *v*) in polar phases, whereas polar analytes have higher retention (positive *s*, *a*, and *b*) for the same phase. This is opposite for non-polar stationary phases. The coefficient values corresponding to the new C18-amide column did not follow the typical trend observed with the other three columns, which indicates that a different degree of interactions is predominantly taking place. The interactions observed for the new C18-amide are discussed below.

From equation 1, the *e*-term represents the interactions through nonbonding n- and p-electrons [27]. All stationary phases have a positive interaction through nonbonding n- and p-electrons, indicating that the stationary phase has several nonbonding electrons; this is in agreement with other reported studies [11, 27, 37].

The nonbonding n- and p-electrons interactions with the silica stationary phase arise from the nonbonding electrons from the oxygen on the silica. The positive interaction present in the amide phases can account for the non-bonding electrons of the nitrogen. The silica support on C18 phase also provides for nonbonding electrons interactions. The *e*-term accounts for some of the polarizability/induction effect, similar to the *s*-term, which results in a chemical overlap between these two terms; therefore, no simple interpretation can be provided [22].

The *s*-term measures the system ability to engage in dipole–dipole interactions [27]; such interactions are typical of the polar stationary phases. As seen in Figure 2, the magnitude of the *s*-term for the terminal-amide phase is higher than that of silica (*s*-term of 0.813 vs. 0.198). In the case of the terminal-amide phase, one can visualize a combination of dipolar interactions attributed to the exposed amide group and to the unreacted residual hydroxyl groups on the silica surface; the silica column only provides the silanol groups. In the case of the C18 and the C18-amide phases, containing the non-polar aliphatic moieties, the *s*-term was negative indicating a stronger interaction with the mobile phase. Such a dipole-dipole interaction can be attributed to the presence of methanol in the mobile phase, since CO_2 is non-polar. Considering the values of the system constant, one may infer that the dipolar interactions with the C18-amide phase are stronger (i.e., the *s*-term slightly less negative) than that for the C18 phase (-0.129 vs. -0.257); however, there is not a statistical difference (95% confidence) between the two values.

The *a*-term represents the ability to donate a lone pair of electrons or accept a H-bond [27]. For the C18 stationary phase, the *a*-term is statistically (95% confidence) indistinguishable from zero. Should there be any interaction, it would come from the methanol-containing mobile phase. The other three stationary phases

249 (i.e., terminal-amide, C18-amide, and silica) have a higher ability to donate a lone
250 pair of electrons than the mobile phase, reflected in the relatively large positive value
251 of the *a*-term. This interaction is stronger for the amide phases than for silica, with an
252 *a*-term of 2.649 for the terminal-amide, 1.793 for the C18-amide, and 1.426 for silica.
253 The electron density of the amide group allows it to act as a H-bond acceptor. The
254 electron donating or H-bond accepting abilities of the C18-amide column appeared to
255 be in between that of the terminal-amide and the silica phase.

256 The *b*-term measures the ability to accept a lone pair of electrons or donate a
257 H-bond [27]. The silica and terminal-amide phases have a higher ability to accept a
258 lone pair of electrons. The silanol groups present in the silica phase can donate H-
259 bonds, which is reflected by the large positive *b*-term (1.539). In the case of the
260 terminal-amide stationary phase the N–H dipoles allow for the amide to donate H-
261 bonds; this is also appreciated by the positive *b*-term of 0.884. For C18 and C18-
262 amide phases, *b*-term of -0.297 and -0.450 respectively, the electron deficient mobile
263 phase has a stronger propensity of accepting a lone pair of electrons. It is reasonable
264 to assume that the C18-amide phase has a more negative *b*-term because of the
265 additional carbon chain extended spacer between the silica surface and the amide
266 moiety; this under layer may decrease the amide H-bond donating ability.
267 Furthermore, the C18 phase has a lower surface coverage than the C18-amide (2.5
268 μmol/m^2 vs. 3 μmol/m^2), meaning that it is possible to have a higher number of
269 accessible –OH, capable of H-bonding, on the silica support of the C18 phase than on
270 that of the C18-amide phase. The uncertainty on the *b*-term obtained in the C-18
271 column, however, does not make it different (95% confidence) from that of the C18-
272 amide phase.

The *v*-term measures the hydrophobic interaction between the mobile phase and the stationary phase [27]; typically, reverse phase type of interactions show as positive values while normal phase interactions give a negative *v*-value. Of the four phases studied, those containing aliphatic groups showed very favorable hydrophobic interactions. C18 showed the strongest interaction in comparison to any of the other stationary phases under study, resulting in the highest value for the *v*-term (i.e., 0.713). This was followed by the C18-amide phase with a value of 0.608. When comparing these two values, one can rationalize that the polar amide groups embedded in the C18-amide phase are responsible for the lower *v*-value observed; nevertheless, hydrophobic interactions are favorable in this phase. Still, the *v*-term is not statistically different (95% confidence) between these two phases. The terminal-amide and the silica columns, on the other hand, showed a negative *v*-value, which favors the behavior of normal phase type of interaction due to the polar nature of the phases. This indicates that the mobile phase dispersive interactions under the SFC conditions used are stronger than those of silica or the terminal-amide phases.

3.3 Selectivity for polar compounds

A group of five small drug-like molecules in a test mixture was used to examine the separation ability of all the columns studied under a given set of isocratic conditions. The mixture consisted of caffeine, uracil, and three widely used pharmaceuticals, nifedipine, bupropion, and naproxen; the structures of these compounds are shown in Figure S2. The compounds were separated under similar supercritical fluid mobile phase conditions; typical chromatograms for the separation of the five-component mixture on each column are shown in Figure 3. The efficiency of the separation can be improved by exploring different experimental condition (e.g.,

298 mobile phase additive, solvent strength) and this can be performed on any given
299 application for each chromatographic column. The main focus of the work here,
300 however, was to compare the selectivity of the different chromatographic columns
301 and we did not attempt to optimize separation efficiency. In our experiments, all the
302 experimental variables were held constant, which allows for the appropriate
303 selectivity comparison among the different columns via the LSER method. We also
304 point out that the linear velocity for each column may have not been at its optimum,
305 which may affect the efficiency. Not surprising, the selectivity of the stationary
306 phases under study is clearly different.

307 Under the chromatographic conditions used, the compounds bupropion,
308 caffeine, and uracil were not baseline resolved by the C18 stationary phase, while
309 naproxen and nifedipine co-eluted. In the case of the silica column, a much longer
310 time was required to separate the components at a methanol co-solvent concentration
311 of 5%. The terminal-amide stationary phase was able to separate caffeine, naproxen,
312 and nifedipine under 12 minutes; however, there was a strong retention for uracil (not
313 shown in the chromatogram), while bupropion eluted with the void volume. The
314 solvent strength of 5% methanol was not enough to elute uracil from the column. This
315 is indicative of a very strong polar interaction with the terminal-amide phase (i.e.,
316 large *a*-term in the LSER model). Uracil was eluted when the co-solvent was
317 increased to a concentration of 15%. In comparison to the silica column, the elution
318 time of caffeine and naproxen was transposed in the terminal-amide phase. The
319 addition of the amide to the silica surface, through a short hydrocarbon linker,
320 provides different interactions of these two solutes; however, strong polar interactions
321 prevail as observed by the strong retention of uracil. In the case of the C18-amide
322 stationary phase, baseline resolved peaks are observed in the chromatogram and all

drug-like compounds were separated under 5.5 minutes. The combined interactions of the C18-amide phase provided selectivity that is different to the other three phases. The log *k* obtained with the C18-amide plotted against the log *k* obtained with the other columns did not show a strong linear relationship (see Figure S3). When comparing both amide phases, the elution order for caffeine, naproxen, and nifedipine is similar; however, for the terminal-amide bupropion elutes with the void volume, while uracil interacts more strongly with this stationary phase.

4 Concluding Remarks

The LSER model brings some insight to the selectivity observed in the separation of the five components in a drug-like mixture by the four stationary phases under study. It appears that the C18-amide stationary phase has characteristics that are favorable for SFC. Although the overall interactions towards polar compounds appear to be weaker than those of silica or terminal-amide columns, it showed good selectivity in the separation of polar compounds. The combined interactions of the C18-amide column provided for baseline resolution of all the polar components in a probe mixture. The H-bond accepting ability of the embedded amide group showed a very favorable positive interaction for polar compounds, notwithstanding the hydrophobic interactions provided by the hydrocarbon backbone. The C18-amide column provided alternate separation selectivity with an advantageous faster analysis time when compared with the terminal-amide and the silica columns.

Acknowledgements

We thank Alan P. McKeown (Advanced Chromatography Technologies Ltd.), Geoff Faden and Edward Faden (MAC-MOD Analytical, Inc.) for the generous donation of

the ACE® C18-amide column. We also acknowledge the financial support for this work by the U.S.A. National Science Foundation (CHE 1508105). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

The authors have declared no conflict of interest

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461

462 **Figure captions**

463 **Figure 1**. A) Scatter plot of the predicted log *k* vs. the observed log *k* of compounds
464 marked with an asterisk in Table 1 for the four stationary phases studied. B) Residual
465 plot of the Scatter plot of the predicted log *k* vs. the observed log *k*.
466

467 **Figure. 2**. Coefficient values obtained from the LSER model for the stationary phases
468 studied.
469

Figure 3. Chromatograms showing the separation of (1) caffeine, (2) bupropion, (3) uracil, (4) naproxen, and (5) nifedipine in a sample mixture using four different chromatographic columns under SFC conditions: C18-amide, terminal-amide, silica, and C18. Chromatographic conditions and columns are described in Section 2.2.

Table 1. Chromatographic solutes and LSER descriptors[a)]

No.	Compound	E[b)]	S[c)]	A[d)]	B[e)]	V[f)]
1	Propylbenzene	0.604	0.50	0.00	0.15	1.1391
2	Butylbenzene	0.600	0.51	0.00	0.15	1.2800
3	Benzaldehyde	0.820	1.00	0.00	0.39	0.8730
4	Naphthalene	1.340	0.92	0.00	0.20	1.0854
5	Biphenyl	1.360	0.99	0.00	0.26	1.3242
6	Benzyl alcohol	0.803	0.87	0.39	0.56	0.9160
7	Phenol	0.805	0.89	0.60	0.30	0.7751
8	Benzoic acid	0.730	0.90	0.59	0.40	0.9317
9	Aniline*	0.955	0.96	0.26	0.50	0.8162
10	N,N-Dimethylaniline	0.957	0.84	0.00	0.47	1.0980
11	Ibuprofen	0.860	0.84	0.59	0.50	1.7800
12	Caffeine	1.500	1.60	0.00	1.35	1.3630
13	o-cresol	0.840	0.86	0.52	0.30	0.9160
14	p-cresol	0.820	0.87	0.57	0.31	0.9160
15	m-cresol	0.822	0.88	0.57	0.34	0.9160
16	Phloroglucinol	1.355	1.12	1.40	0.82	0.8925
17	Bromobenzene	0.882	0.73	0.00	0.09	0.8910
18	Chlorobenzene	0.718	0.65	0.00	0.07	0.8288
19	Nitrobenzene	0.871	1.11	0.00	0.28	0.8906
20	Anisole*	0.708	0.75	0.00	0.29	0.9160
21	Naphthoic acid	1.200	1.27	0.52	0.48	1.3007
22	Acetophenone	0.818	1.01	0.00	0.48	1.0139
23	2,4-Dimethylphenol*	0.840	0.80	0.53	0.39	1.0570
24	2,6-Dimethylphenol	0.860	0.79	0.39	0.39	1.0570
25	p-nitrophenol	1.070	1.72	0.82	0.26	0.9490
26	o-nitrophenol*	1.045	1.05	0.05	0.37	0.9490
27	m-nitrophenol	1.050	1.57	0.79	0.23	0.9490
28	Benzophenone	1.447	1.5	0	0.5	1.481

a) Compounds marked with an asterisk are the solutes used for the assessment of prediction for the model.

b) E is the excess molar refraction

c) S is the solute dipolarity/polarizability

d) A is the solute overall acidity

e) B is the solute overall basicity

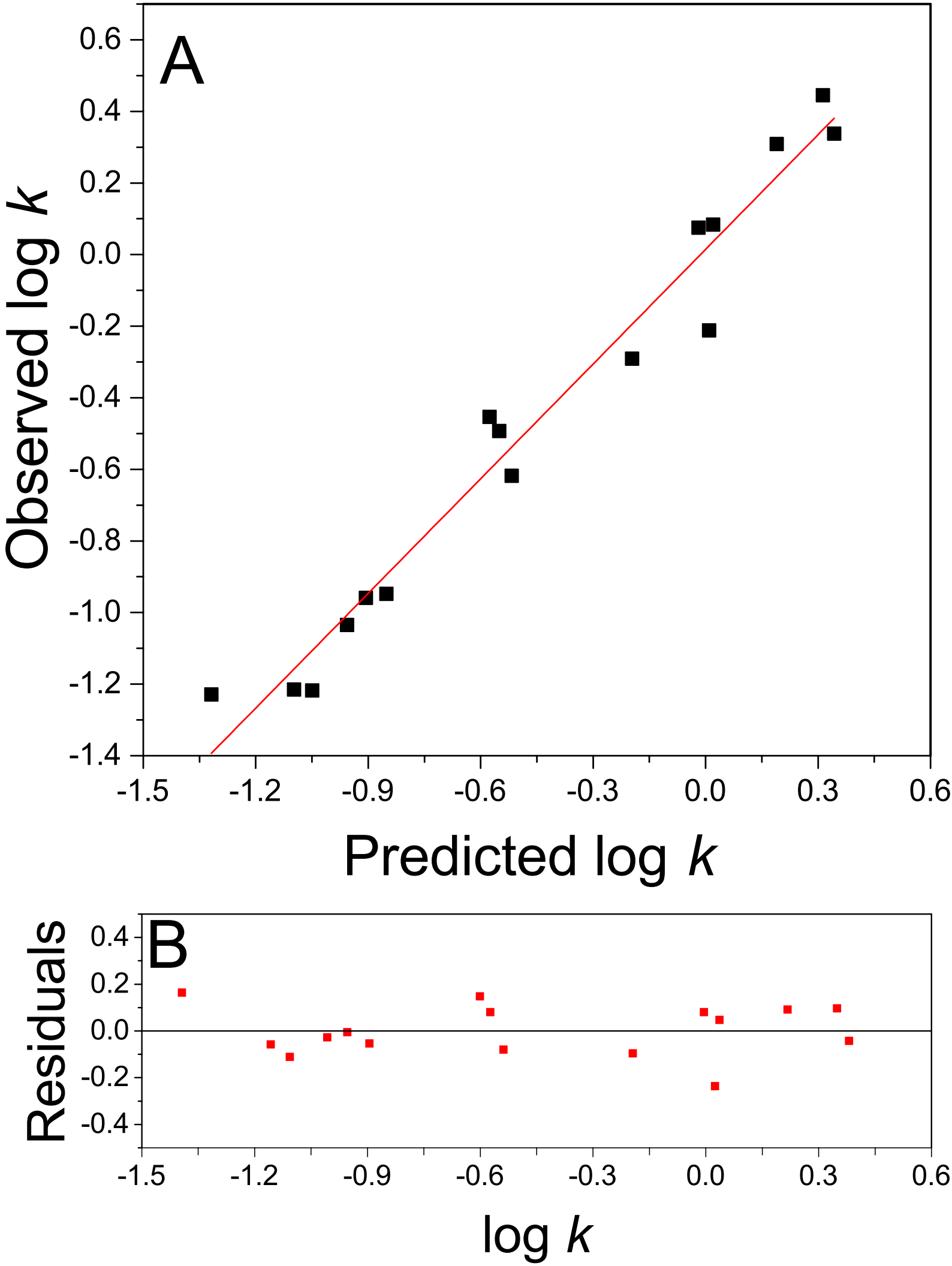
f) V is the McGowan characteristic volume

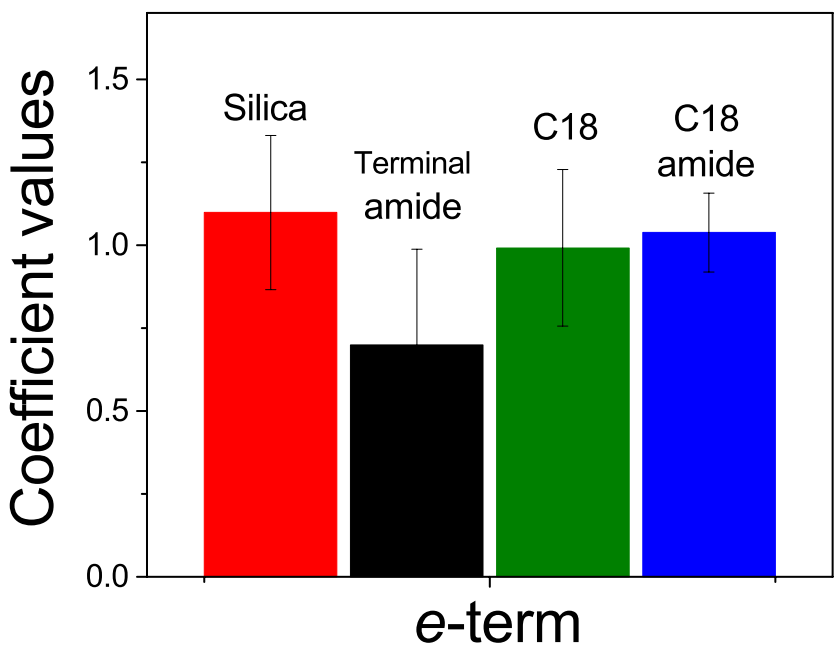
Table 2. LSER constants[a)] and statistics[b)] for the stationary phases studied

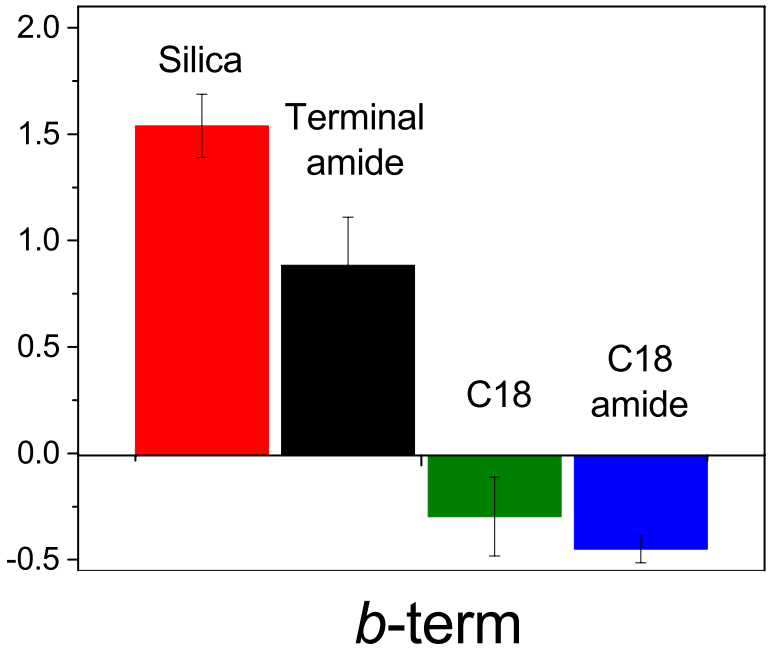
Stationary Phase	c	e	s	a	b	v	n	R	SE	F
Silica	-1.254	1.098	0.198	1.426	1.539	-1.116	22	0.985	0.143	$F_{(5,17;0.05)} = 106$
Terminal amide	-2.241	0.699	0.813	2.649	0.884	-0.367	22	0.984	0.170	$F_{(5,17;0.05)} = 98$
C18	-2.211	0.992	-0.257	-0.055	-0.297	0.713	24	0.865	0.187	$F_{(5,20;0.05)} = 11$
C18-amide	-1.697	1.038	-0.129	1.793	-0.450	0.608	21	0.996	0.058	$F_{(5,16;0.05)} = 365$

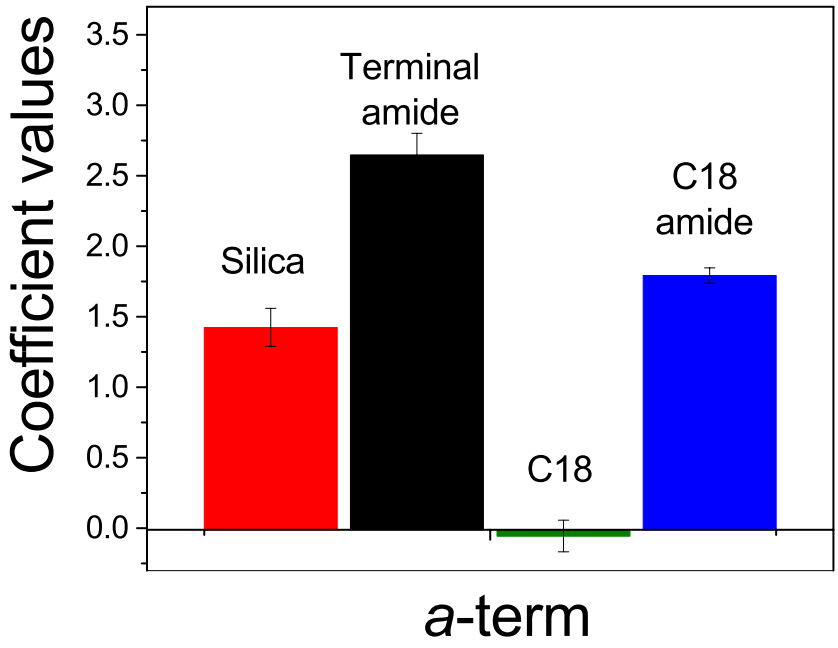
a) As defined in text

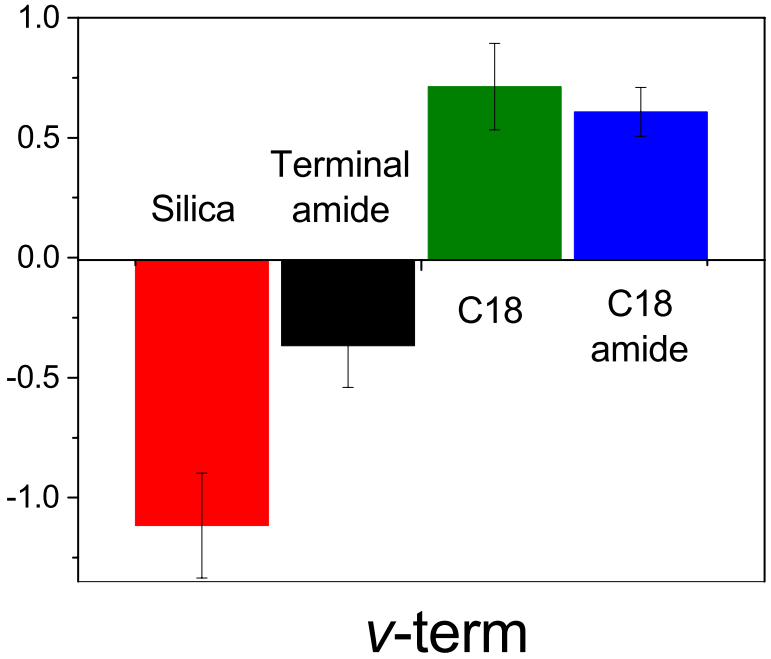
b) n is the number of solutes considered in the multilinear regression; R is the multiple correlation coefficients; SE is the standard error of the estimate; F is the Fisher F-statistics test.

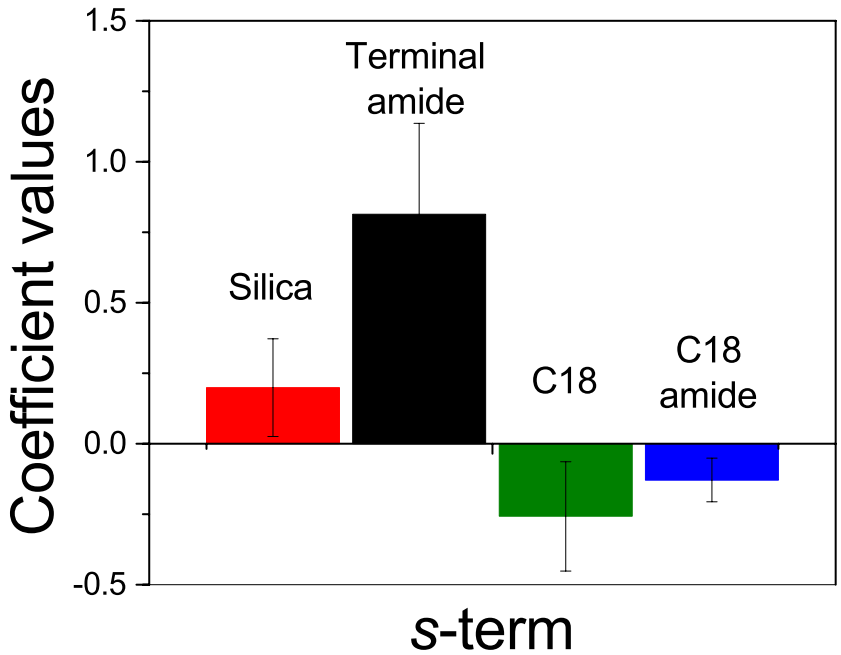

A
Observed log k
Predicted log k
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.5
-1.2
-0.9
-0.6
-0.3
0.0
0.3
0.6
B
Residuals
log k
0.4
0.2
0.0
-0.2
-0.4


Coefficient values
1.5
1.0
0.5
0.0
Silica
Terminal amide
C18
C18 amide
e-term


2.0
1.5
1.0
0.5
0.0
-0.5
Silica
Terminal amide
C18
C18 amide
b-term


Coefficient values
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Terminal amide
Silica
C18
C18 amide
a-term


1.0
0.5
0.0
-0.5
-1.0
Silica
Terminal amide
C18
C18 amide
v-term


Coefficient values
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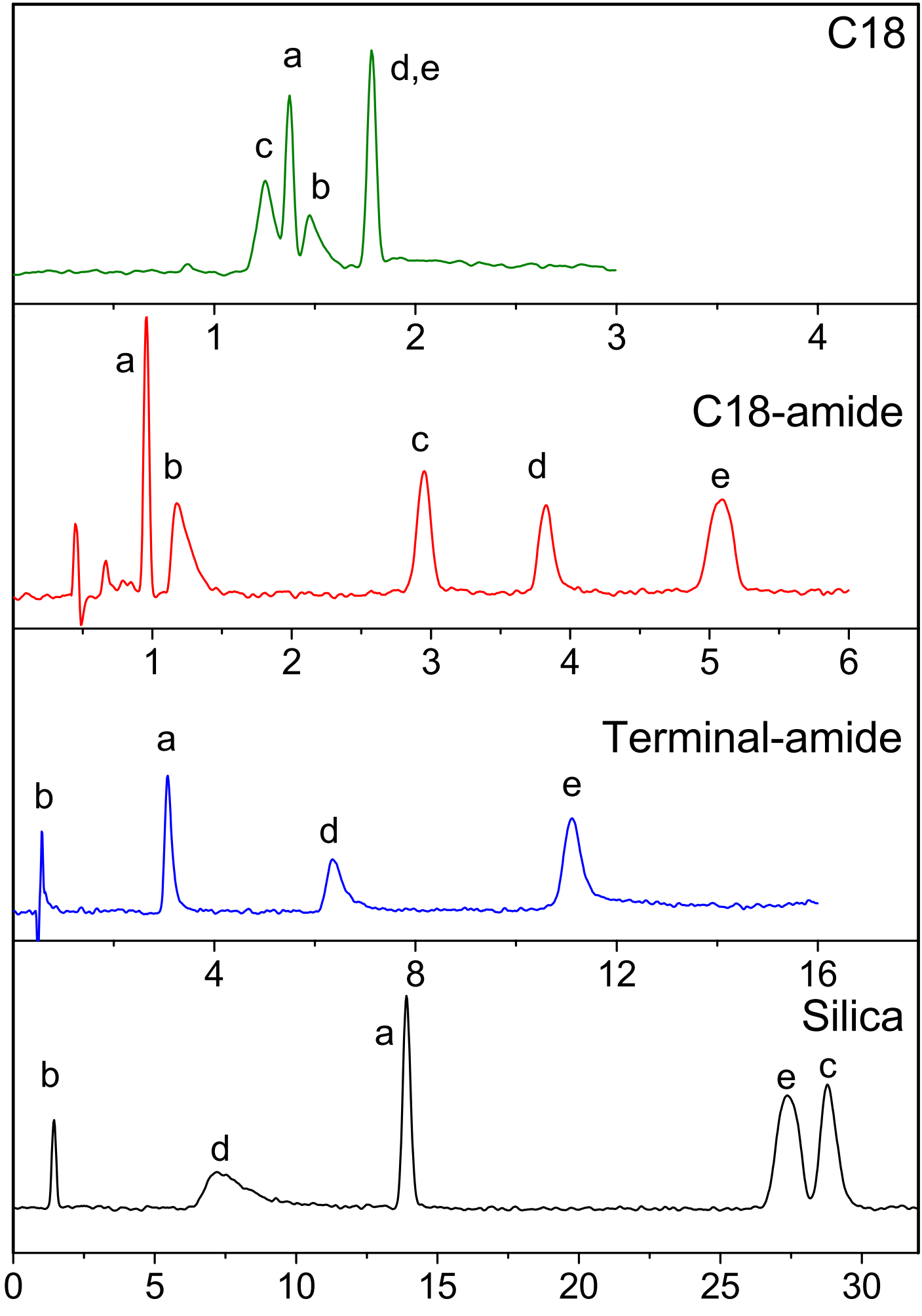
0.5
0.0
-0.5
Terminal amide
Silica
C18
C18 amide
s-term


C18
a
d,e
c
b
1
2
3
4
a
C18-amide
b
c
d
e
1
2
3
4
5
6
Terminal-amide
a
b
d
e
4
8
12
16
Silica
b
a
e
c
d
0
5
10
15
20
25
30
Time (min)