



HAL
open science

Unravelling the effects of mobile phase additives in supercritical fluid chromatography-Part II: Adsorption on the stationary phase

Caroline West, Elise Lemasson

► **To cite this version:**

Caroline West, Elise Lemasson. Unravelling the effects of mobile phase additives in supercritical fluid chromatography-Part II: Adsorption on the stationary phase. *Journal of Chromatography A*, 2019, 1593, pp.135 - 146. 10.1016/j.chroma.2019.02.002 . hal-03486974

HAL Id: hal-03486974

<https://hal.science/hal-03486974>

Submitted on 20 Dec 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Unravelling the effects of mobile phase additives in supercritical fluid** 2 **chromatography. Part II: Adsorption on the stationary phase**

3
4 Caroline West ^{*1}, Elise Lemasson¹

5 1. Univ. Orleans, CNRS, Institut de Chimie Organique et Analytique (ICOA), UMR 7311,
6 B.P. 6759, rue de Chartres, F-45067 Orléans cedex 2, France.

7
8 Corresponding author:

9 Tel.: (+33)238 494 778

Fax.: (+33)238 417 281

10 caroline.west@univ-orleans.fr

11 12 **Abstract**

13
14 The mobile phases employed in current practice of supercritical fluid chromatography (SFC)
15 are usually composed of a mixture of pressurized carbon dioxide and a co-solvent. The co-
16 solvent is most often an alcohol and may contain a third component in small proportions,
17 called an additive (acid, base or salt). In the first part of this series, the effects of mobile
18 phase additives on the polarity and apparent pH of the mobile phase were explored. In the
19 present paper, we examine the effects pertaining to adsorption of additives on the stationary
20 phase. Ammonium acetate was selected as a representative case, because it is often
21 employed in current practice. To favour its solubility and further improve chromatographic
22 quality, a small portion of water is also advocated. First, the equilibration time is observed to
23 be largely increased in the presence of an additive, especially when mobile phase
24 compositions containing only low proportions of methanol co-solvent are employed.
25 Secondly, the effects of ammonium acetate are more thoroughly assessed with a modified
26 version of the solvation parameter model (five Abraham descriptors and two descriptors to
27 take account of positive and negative charges on ionizable species). On a hybrid silica
28 stationary phase (ACQUITY UPC² BEH), the effects of increasing concentration of
29 ammonium acetate (0 to 25 mM in the methanol co-solvent) are investigated. The retention
30 of acidic species is the most strongly affected, with a continuous retention increase when
31 additive concentration increases. The retention of basic and neutral species is also
32 moderately affected. Then thirty-two stationary phases based on sub-2 µm totally porous
33 particles or sub-3 µm superficially porous particles are characterized with and without 20 mM
34 ammonium acetate and 2% water in the methanol co-solvent. The effects of adsorbed
35 additive on the interaction capabilities are discussed. Finally, the interest of introducing the
36 additive in the dilution solvent, a method that was sometimes recommended to simplify the
37 workflow, is also discussed with a model basic compound.

38
39
40 **Keywords:** adsorption; ionic interactions; selectivity; solvation parameter model;
41 supercritical fluid chromatography.

43 1. Introduction

44

45 Because the solvation strength of neat carbon dioxide, the main component of the
46 mobile phases employed in supercritical fluid chromatography (SFC), is rather low, a
47 co-solvent is usually introduced [1,2]. Typical proportions employed in most
48 applications range from 1 to 50% [3], although recent papers have shown higher
49 proportions to favour the solubility of difficult analytes and extend the polarity range
50 of analytes amenable to the technique [4,5]. In most current SFC research, the co-
51 solvent is an alcohol (methanol in a majority of cases, ethanol or isopropanol). To
52 further facilitate the elution of most polar species and ionizable analytes with good
53 peak shapes, a third component is often introduced in the co-solvent in smaller
54 proportions (typically 0.1 to 1%, or 10-20 mM in the co-solvent), termed “additive”.
55 For chiral separations, the additive is also known to affect enantioselectivity [6–8].
56 Most employed additives are acids (like acetic acid, formic acid, trifluoroacetic acid
57 [8,9]), bases (like isopropylamine, diethylamine or other amines [10–12]), mixtures of
58 acids and bases [13,14] or salts (ammonium acetate, ammonium formate,
59 ammonium hydroxide or other alkylammonium hydroxide salts [15]). Basic additives
60 are also supposedly limiting unwanted reaction between basic analytes and carbon
61 dioxide [16]. Water is also advocated, sometimes in combination with another
62 additive [10,16,17], especially for salts as water would limit the risks of salt
63 precipitation. In some occasions, it was also recommended to introduce the additive
64 in the analyte dilution solvent [18,19], as this was supposed to favour ion pairing with
65 ionizable analytes, thereby improving their elution.

66

67 It was long speculated that the additive has an effect both on mobile phase and
68 stationary phase polarity. In the first part of these series [20], we have shown that
69 additives employed at usual concentration ranges have no significant effect on
70 mobile phase polarity but have some effect on mobile phase apparent acidity.
71 Indeed, mobile phases composed of carbon dioxide and an alcohol co-solvent have
72 an apparent pH of about 5, which is shifting to slightly more acidic values in the
73 course of a mobile phase gradient, when the alcohol proportion increases. In this
74 report, it was concluded that acidic additives may further decrease the apparent pH
75 of the SFC mobile phase, while basic additives and salts only slightly increased the

76 apparent pH but essentially afforded some buffering of the apparent pH over the
77 course of an elution gradient.

78 Adsorption of mobile phase components on the stationary phase was demonstrated
79 long ago by Strubinger et al. [21,22] and Berger and Deye [23]. The adsorption of
80 carbon dioxide and co-solvent is rather well documented. For instance, a few recent
81 significant studies can be found in the works of Rajendran [24] and Glene et al. [25–
82 27], which were principally focused on adsorption of the co-solvent. Unfortunately,
83 the adsorption of additives was not examined so comprehensively. A recent study of
84 Speybourck et al. [28] investigated the effects of unusually high concentrations of
85 isopropylamine on enantioseparations with polysaccharide chiral stationary phases
86 and demonstrated that enantioselectivity could be fine-tuned by varying the
87 concentration of additive.

88 Additive adsorption on the stationary phase and its consequences on retention
89 behaviour is our main interest in this paper. For this purpose, we focused mainly on
90 one additive combination, comprising water and ammonium acetate. This
91 combination has proven to allow for improved mass spectrometric detection and
92 chromatography through (i) larger proportion of analytes successfully eluted, (ii)
93 better peak shapes (iii) improved efficiency and (iv) improved ionisation with
94 electrospray ionisation source [29]. The interest in combining water with a salt
95 additive is also that the former is favouring solubility of the latter, thus limiting the
96 risks of precipitation when the solvent is mixed to pressurized CO₂, and upon CO₂
97 depressurization after the back-pressure regulator, when the cooling effect is
98 reducing the solubility of salts. To provide the most complete picture of additive
99 adsorption, a large number of stationary phases (thirty-two) are included in this study,
100 comprising both polar and non-polar surface chemistries. Most of these stationary
101 phases were previously characterized in SFC with mobile phases comprising only
102 CO₂ and methanol, but no additive [30]. Linear solvation energy relationships (LSER)
103 are employed to deconvolute the changes in stationary phase interaction capabilities
104 when additive is adsorbed on the surface. LSER methodology was previously
105 employed to qualify the changes occurring in SFC when additives were introduced in
106 the mobile phase [6], but previous works employed only neutral species, while we
107 expect that ionizable species should be most significantly affected by an additive.
108 One particular silica stationary phase is investigated in more details to observe the
109 changes when the additive concentration is progressively increased. The

110 consequences of using an additive on column equilibration are also discussed.
111 Finally, the effects of introducing an additive solely in the injection solvent to improve
112 the peak shape of basic analytes is also examined.

113
114

115 **2. Theory**

116

117 The usual LSER equation employed in chromatography, based on Abraham
118 descriptors, is as follows [31]:

119

$$120 \log k = c + eE + sS + aA + bB + vV \quad (1)$$

121

122 In this equation, capital letters represent the solute descriptors, related to particular
123 interaction properties, while lower case letters represent the system constants,
124 related to the complementary effect of the phases on these interactions. c is the
125 model intercept term and is dominated by the phase ratio. E is the excess molar
126 refraction (calculated from the refractive index of the molecule) and models
127 polarizability contributions from n and π electrons; S is the solute dipolarity /
128 polarizability; A and B are the solute overall hydrogen-bond acidity and basicity; V is
129 the McGowan characteristic volume in units of $\text{cm}^3 \text{mol}^{-1}/100$.

130 To take account of ionic interactions with anionic and cationic species, we have
131 previously proposed to extend Eq. (1) with two additional terms, as follows [32,33]:

132

$$133 \log k = c + eE + sS + aA + bB + vV + d^-D^- + d^+D^+ \quad (2)$$

134

135 where D^- represents the negative charge carried by anionic and zwitterionic species,
136 and D^+ represents the positive charge carried by cationic and zwitterionic species,
137 according to the following equations:

138

$$139 D^- = \frac{10^{(pH^* - pK^*)}}{1 + 10^{(pH^* - pK^*)}} \quad (3)$$

$$140 D^+ = \frac{10^{(pK^* - pH^*)}}{1 + 10^{(pK^* - pH^*)}} \quad (4)$$

141

142 For neutral species, D^- and D^+ are zero so that Eq. (2) reverts to Eq. (1).

143 Judging from the results presented in the first paper in this series [20], the apparent
144 pH should be around 5 in SFC with carbon dioxide-methanol mobile phases, with or
145 without ammonium acetate and water. For the purpose of simplicity and for want of a
146 better solution, we may simply consider aqueous pK values. Indeed, there are
147 currently no established methods to measure the pK values in pressurized CO₂-
148 solvent mixtures.

149

150 The system constants (e , s , a , b , v , d' and d^+), obtained through a multilinear
151 regression of the retention data for a certain number of solutes with known
152 descriptors, reflect the magnitude of difference for that particular property between
153 the mobile and stationary phases. Thus, if a particular coefficient is numerically large,
154 then any solute having the complementary property will interact very strongly with
155 either the mobile phase (if the coefficient is negative) or the stationary phase (if the
156 coefficient is positive). The system constants facilitate the comparison of the
157 separation characteristics of different stationary phases and, in the present case, will
158 allow to identify the changes occurring when an additive is present in the mobile
159 phase.

160

161

162 **3. Material and methods**

163

164 **3.1. Stationary phases**

165

166 All stationary phases used in this study are commercially available. The names and
167 known properties of the columns used are presented in Table 1.

168

169 **3.2. Chemicals**

170

171 The solvent used was HPLC-grade methanol (MeOH) provided by VWR (Fontenay-
172 sous-Bois, France). Carbon dioxide of industrial grade 99.5% was provided by Air
173 Liquide (France). Solutions of all test compounds were prepared in MeOH. 114 test

174 compounds (Table S1), comprising 90 analytes that should be charge-neutral in the
175 present operating conditions, and 24 species that may be partly or completely ionized
176 (anionic or cationic form), were obtained from a range of suppliers. More solutes
177 were initially analyzed with all chromatographic columns, but some of them suffered
178 of very low retention on some columns (for instance, hydrophobic species on polar
179 stationary phases), or excessive retention in the absence of an additive, thus only
180 those compounds that were eluted with measurable retention on a large majority of
181 the columns were finally retained in the calculations. For all tests without additive, the
182 number of analytes retained in the calculation is only 109, as some of the most basic
183 species could not be eluted from many columns.

184 The Abraham solute descriptors (E, S, A, B, V) used for LSERs with Eq. (1) were
185 extracted from an in-house database established from all available literature on the
186 solvation parameter model (Table S1). The two additional descriptors (D^- and D^+)
187 were calculated based on Eqs. (3) and (4), and based on aqueous pK values
188 determined with Chemicalize freeware (<http://www.chemicalize.org/>). The series of
189 test-compounds has been selected by observing the requirements of a good LSER
190 analysis [31]. The compounds were chosen so as to provide a uniform distribution of
191 each descriptor within a wide enough space and absence of cross-correlation
192 among the descriptors was checked. Full description of the set of analytes can be
193 found in previous works [33].

194

195 **3.3. Chromatographic system and conditions**

196

197 Chromatographic separations were carried out using an ACQUITY Ultra Performance
198 Convergence ChromatographyTM (UPC²) system from Waters (Millford, MA, USA).

199 The system was equipped with a binary solvent delivery pump compatible with
200 mobile phase flow rates up to 4 mL/min and pressures up to 414 bar, an
201 autosampler, a backpressure regulator, a column oven compatible with 150 mm
202 length columns, and a photodiode-array (PDA) detector. Chromatograms were
203 recorded with Empower® 3 software.

204 The detection wavelength was 210 nm.

205 The mobile phase used in this study is always CO₂-MeOH (v/v) with or without an
206 additive. The additive was prepared as a 1 M solution of ammonium acetate in
207 distilled and deionized water, which was diluted with methanol to the desired

208 concentration (from 2 to 25 mM). Thus mobile phase composition comprises both
209 water and ammonium acetate in varying but correlated concentrations. When the
210 mobile phase composition was changed, the new composition was let to equilibrate
211 until the breakthrough curve followed by a stable baseline were observed, which may
212 vary between one minute and two hours depending on mobile phase composition
213 and flow rate, as will be further detailed in the discussion section.

214 Flow rate was 1 mL min⁻¹ for sub-2 µm fully porous particles (FPP) columns and 3
215 mL min⁻¹ for sub-3 µm superficially porous particles (SPP) columns. The average
216 internal pressure was thus in a similar range on all columns. The oven temperature
217 was set at 25°C and the outlet pressure was maintained at 150 bar for all columns.
218 Injection volume was 0.5 µL for all compounds on all columns. Although this would
219 result in different dilution depending on column dimensions, this should not affect the
220 measurements done in this study as only retention times were considered, not
221 efficiency.

222 Retention factors (k) were calculated based on the retention time t_R , determined
223 using the peak maximum (even when tailing occurred) and on the hold-up time t_0
224 measured on the first negative peak due to the unretained dilution solvent.

225

226 **2.4. Data analysis**

227

228 Multiple linear regressions (MLR) were performed using XLStat 2015.2.02 software
229 (Addinsoft, New York, NY). The quality of the MLR fits was estimated using the
230 adjusted determination coefficient (R^2), standard error in the estimate (SE) and
231 Fisher F statistic. The statistical significance of individual coefficients was evaluated
232 with the 95% confidence intervals.

233

234 **4. Results and discussion**

235

236 **4.1. Adsorption assessed with breakthrough curves**

237

238 For this series of experiments, only the ACQUITY UPC² BEH column, a hybrid silica
239 stationary phase, was used. First, the column was equilibrated with neat CO₂ until a
240 stable baseline could be observed, then a step gradient of methanol in varying

241 proportions, with or without additive (20 mM ammonium acetate and 2% water), was
242 applied. UV detection was employed to follow the variations of the baseline. When
243 only methanol was used, whatever the proportion in CO₂, the equilibration was
244 always very fast as indicated by a sharp increase of UV absorbance after the dwell
245 volume (breakthrough of methanol) immediately followed by a stable baseline.
246 However, when the additive was present, after the first sharp increase of UV
247 absorbance, the baseline was not immediately linear, and could take a very long time
248 before reaching a stable, flat state. An extreme example is shown in Figure 1, when
249 only 5% methanol was employed, and the complete equilibration required about 75
250 minutes, which represents more than a 100 column volumes. The apparently erratic
251 shape of the baseline was actually well reproducible, as the experiment was
252 repeated three times and systematically showed this two-humps, camel-like
253 appearance. Probably, because the additives were present at a very low
254 concentration in the overall mobile phase composition (1 mM ammonium acetate and
255 0.1% water), it took a long time for them to cover the surface of the stationary phase
256 completely to reach a stable and homogeneous layer. These strange curves make it
257 difficult to measure the amount of additive deposited on the stationary phase. Indeed,
258 such measurement is usually based on a “regular” breakthrough curve, considering
259 the time to saturate the stationary phase prior to breakthrough. However, in the
260 present case, the stationary phase is probably not saturated when the breakthrough
261 occurs and further equilibration is occurring.

262 Higher proportions of methanol yielded shorter and shorter equilibration times, with
263 the two humps rapidly fading into one, but with irregular baseline lasting several
264 minutes afterwards (see Figure S1 in supplementary material). For instance, a step
265 gradient from 0 to 10% co-solvent comprising additive took 42 minutes to equilibrate
266 (about 60 column volumes); step gradient from 0 to 20% took 22 minutes (about 30
267 column volumes); further increases (0 to 30%, 0 to 40% or 0 to 50%) took about 19,
268 18 and 16 minutes respectively. Furthermore, when the column had first been
269 equilibrated with 5% co-solvent comprising additive, a further increase from 5 to 10%
270 co-solvent took about 14 minutes for a full equilibration; moving from 10 to 15% took
271 7 minutes; then only 4 minutes were sufficient to equilibrate from 15 to 20%, etc.

272 Thus when the stationary phase has already been covered with adsorbed methanol
273 and additives, further increases in solvent proportion allow to reach the equilibrium at
274 a faster rate.

275 These observations should also be related to previous experiments from Berger and
276 Deye [23]. When they measured the surface coverage of different stationary phases
277 with acidic or basic additives, they concluded that the amount of additive adsorbed
278 on the stationary phase was independent of its concentration in the mobile phase but
279 was depending on the solvent proportion. Indeed, the adsorbed quantity was the
280 highest when the co-solvent proportion was the lowest. It then seems logical that the
281 layer of adsorbed mobile phase components should take more time to equilibrate
282 when the total amount adsorbed is the highest, that is to say at low solvent
283 proportions.

284 This observation also has a direct practical consequence. Elution gradients in SFC
285 often start at low co-solvent proportions (typically 2-5%). When an additive is present
286 in the co-solvent, it is advisable to first equilibrate the column with large proportions
287 of co-solvent, so as to saturate the stationary phase with co-solvent and additive at a
288 fast rate, then decrease the proportion of co-solvent down to the desired starting
289 composition [34]. Judging from the time required to equilibrate the column with
290 additives, it may be inferred that equally long times (or perhaps longer times) are
291 required to remove the additives from the stationary phase. Depending on the type of
292 additive employed, other fluid compositions may be used to wash the additives from
293 the column (acid wash to remove a base and vice-versa) [23].

294

295 ***4.2. Interaction capabilities assessed with the modified solvation parameter*** 296 ***model***

297

298 We have previously developed a classification of stationary phases for SFC use [35].
299 Recently, this classification was upgraded to include ionic interactions with the help
300 of the modified solvation parameter model described in section 2 [30]. In this last
301 version, only ultra-high performance columns were included, comprising now thirty-
302 two stationary phases based on sub-2 μm fully porous particles (FPP) and sub-3 μm
303 superficially porous particles (SPP). The stationary phase chemistries in this
304 selection varies to a great extent, comprising typical polar phases that are most often
305 employed in SFC (bare silica gel, 2-ethylpyridine, propanediol or aminopropyl
306 ligands), other emerging polar phases (2-picoylamine), non-polar phases
307 (endcapped octadecyl-bonded phases) and phases with intermediate polarity
308 (phenyl, pentafluorophenyl and “polar” octadecyl-bonded phases with non-

309 endcapped silanol groups or polar-embedded groups), as far as SFC is concerned.
310 The purpose of including so many stationary phases in the present evaluation was
311 two-fold. Firstly, we wished to observe whether the effects of additive adsorption
312 would be the same to all stationary phase chemistries, or on the contrary would be
313 more significant on polar phases. It was anticipated that a polar additive should
314 adsorb more significantly on a polar surface or polar ligands. Secondly, we wished to
315 observe whether the choice of mobile phase composition (with or without additives)
316 would make a significant change on the general aspect of the classification of
317 stationary phases.

318

319 4.2.1. Effect of different concentrations of additive on a single silica stationary phase

320

321 First, the effects of increasing concentration of ammonium acetate and water were
322 observed solely on the ACQUITY UPC² BEH column that previously served for the
323 column equilibration experiments and which contains a stationary phase of non-
324 bonded hybrid silica. For this purpose, a 1 M aqueous solution of ammonium acetate
325 was diluted in the methanol co-solvent in different ratios, so as to vary concomitantly
326 the concentration of ammonium acetate from 0 to 25 mM and the concentration of
327 water from 0 to 2.5%.

328 Simply observing the variation of retention for the analytes in Table S1 depending on
329 additive concentration (exemplified with three analytes in Figure 2) yielded the
330 following observations:

- 331 - the retention of charge-neutral polar species was slightly increasing with
332 additive concentration;
- 333 - the retention of basic species decreased more or less sharply with small
334 additions of ammonium acetate (compared to no-additive mobile phase), then
335 was rather stable, and finally slightly increased again at the highest additive
336 concentrations. Although not visible in this figure, the first retention decrease
337 was also associated to a significant improvement in peak shape.
- 338 - The retention of acidic species was continuously increasing with additive
339 concentration.

340 These observations are reflected in the system constants of the LSER models
341 calculated at each additive concentration, as appears with the system map in Figure

342 3. Indeed, examining the variation of system constants, and taking account of error
343 bars, it appears that:

- 344 - the retention of hydrophobic compounds should be unaffected by the
345 concentration of additive, as the e and v terms related to dispersive
346 interactions are constant;
- 347 - the retention of polar non-ionizable species faces a slight U-shaped tendency,
348 first decreasing then slightly increasing when additive concentration increases,
349 as reflected by the s , a and b terms (dipole-dipole and hydrogen bonding
350 interactions);
- 351 - the retention of basic ionizable species is mostly identical to polar non-
352 ionizable species (d^+ term related to interactions with cationic species). The
353 initial decrease in retention is not so sharp as was observed in Figure 2.
- 354 - The retention of acidic species is continuously increasing when additive
355 concentration increases, as reflected in the variation of d^- term.

356 The statistically-drawn LSER system map therefore shows that the behaviour
357 exemplified with three analytes in Figure 2 is mostly in accordance with general
358 trends. It is also interesting to note that some of these variations are consistent with
359 previous observations made in hydrophilic interaction liquid chromatography (HILIC
360 mode) [36], with however some significant differences that may be explained by the
361 proportion of water in the HILIC mobile phase, which is usually much larger than in
362 the present case.

363

364 The interpretation may be as follows.

365 When only CO₂ and methanol are present in the mobile phase, some CO₂ and
366 methanol molecules are adsorbed on the polar stationary phase. The analytes may
367 be retained on the stationary phase with a process that is very similar to what is
368 normally accepted in HILIC [37]: they may partition between the mobile phase and the
369 adsorbed layer of mobile phase components, and interact with the stationary phase
370 underneath, thereby combining partition and adsorption mechanisms.

371 When the water and salt are added to the mobile phase, they will also adsorb on the
372 polar hybrid silica, thereby changing the nature of the pseudo-stationary phase (layer
373 of mobile phase components) in contact with the analytes. This should result in an
374 increased layer thickness and in different interaction capabilities. Berger previously
375 hypothesized that the analytes should be unable to displace polar additives from the

376 surface of the stationary phase [38]. Whether this hypothesis is true or not, we may
377 infer that some of the adsorbed additive molecules also interact with the analytes.
378 For charge-neutral species, the introduction of additives causing increased layer
379 thickness (a phenomenon that is well known in HILIC mode) may explain the slowly
380 increasing retention observed, especially for polar analytes as they would
381 preferentially partition into the pseudo-stationary phase layer of methanol and
382 additives. Non-polar analytes should mostly be unaffected by this effect.
383 With the initial introduction of additives (from 0 to 1 mM ammonium acetate), the
384 additive may first shield the analytes from the silanol groups of the stationary phase,
385 which carry partial or total negative charge. As a result, ionizable basic species that
386 may carry a positive charge in the present conditions are less retained on the
387 stationary phase, and some decrease in retention is first observed. On the contrary,
388 acidic species that may carry a negative charge are less repulsed by the stationary
389 phase and increased retention is observed.
390 With further increases in additive concentration (from 1 to 25 mM ammonium
391 acetate), the pseudo-stationary phase now contains water molecules, ammonium
392 ions and acetate ions (in addition to CO₂ and methanol molecules), which may all
393 interact with ionizable analytes. When all silanol groups are shielded, ammonium
394 ions may cause progressively increasing retention of acidic species, while acetate
395 ions would finally contribute to retaining basic species as well.
396 Naturally, mobile phase effects may also occur to contribute to these effects, with
397 possible ion-pairing effects (e.g. between acetate ion and basic species) or changes
398 in the apparent acidity of the mobile phase causing different ionization state of the
399 analytes. The possible effects of additives on analytes shall be further discussed in
400 future works.

401
402 Based on the curves in Figures 2 and 3, it seems that a stable state of the BEH
403 stationary phase was still not reached with 25 mM ammonium acetate, as a plateau
404 cannot be seen on all curves. In previous works carried out on a non-encapped
405 octadecylsiloxane-bonded silica stationary phase (ACQUITY HSS C18 SB), we had
406 observed that 20 mM ammonium acetate and 2% water were sufficient to obtain
407 stable chromatograms for basic drugs (no more retention or peak shape variation)
408 [29]. In other applications with different stationary phases, other optimal
409 concentrations were advocated. Clearly, this “equilibrium concentration” should be

410 highly dependent on the stationary phase but also on the operating conditions. As it
411 would be unreasonable to explore the effect of additive concentration on all 32
412 stationary phases included in the present study, we decided to fix the ammonium
413 acetate and water concentrations to 20 mM and 2% respectively for the following
414 experiments. Higher concentrations are hardly advisable, as they may cause salt
415 precipitation or could be deleterious to mass spectrometric detection.

416

417

418 4.2.2. Effect of a single concentration of additive on 32 different stationary phases

419

420 With the fixed mobile phase composition (20 mM ammonium acetate and 2% water
421 in the methanol co-solvent), all 32 stationary phases were characterized with LSER
422 Eq. (2) to take account of possible changes in the retention of ionizable species.
423 Looking at each stationary phase individually, with or without the additives in the
424 mobile phase, some common patterns can be observed. Full data can be observed in
425 Table 2 while selected histograms are presented in Figure 4.

426 In Figure 4, six stationary phases based on the same silica particles (Accucore
427 superficially porous silica particles) but with different stationary phase chemistries are
428 presented to illustrate the contribution of the surface polarity to the effect of additives.

429

430 First, Accucore Phenyl-hexyl is a typical example of non-polar stationary phase.
431 Indeed, the general retention pattern when no additive is present is consistent with
432 “reversed-phase” mode of retention [35,39]: the terms related to dispersive and π - π
433 interactions (v and e terms) are positive, indicating favourable retention of
434 hydrophobic and aromatic species, while the s , a and b terms related to polar
435 interactions (dipole-dipole and hydrogen bonding) are negative, indicating that polar
436 species are not retained. Interactions with anions are essentially non-significant while
437 some interactions with cationic compounds are observed, which may indicate some
438 accessibility to residual silanol groups. Upon the introduction of additive in the mobile
439 phase, the hydrogen bonding terms (a and b) are even more negative, as may be
440 expected from favourable mobile phase interactions. Non-polar interactions terms are
441 only slightly affected, with small increases of the positive values (e and v). The
442 variation of the d terms, related to interactions with ionic compounds, is the most
443 significant: interactions with anions (d^-) increase from non-significant to positive

444 values, while interactions with cations (d^+) decrease from positive values to non-
445 significant. This is consistent with the covering of residual silanol groups by the
446 additive, as was described with the silica stationary phase: anions that were
447 previously repulsed by silanol groups are no longer repulsed thus more retained, and
448 perhaps attracted by adsorbed ammonium ions; cations that were previously
449 attracted by residual silanol groups are no longer attracted thus less retained.

450
451 Secondly, Accucore Phenyl-X is an example of a polar aromatic phase: the ligand
452 possesses a terminal aromatic group, with an alkyl chain and polar group in the
453 spacer arm. The retention model when no additive is present is significantly different
454 from the retention model of Accucore Phenyl-hexyl. The aromatic terminal group and
455 alkyl spacer arm contribute to strong π - π interactions (e term) and dispersive
456 interactions (v term). The polar group in the spacer arm is contributing to strong
457 hydrogen-bonding interactions with proton donors (positive a term). Silanophilic
458 interactions are however absent, as the other polar interactions are all negative or
459 non-significant (s , b , d' and d^+). When the additive is present in the mobile phase, the
460 most significant changes are observed on s and a terms, which are both increased,
461 suggesting that polar analytes are more retained. Contrary to the non-polar phenyl-
462 hexyl stationary phase, the large concentration of polar embedded groups in the
463 ligands of Accucore Phenyl-X probably contribute to the creation of a dense layer of
464 adsorbed mobile phase components. This is precisely why polar embedded groups
465 were designed in reversed-phase HPLC, with the layer of adsorbed water creating a
466 shielding effect against silanophilic interactions. Ionic interactions (d terms) are also
467 increasing, which is consistent with this hypothesis.

468
469 The third example is that of Accucore PFP, a pentafluorophenyl-bonded silica phase.
470 As was discussed in a previous paper [33], the retention pattern on PFP phases
471 when no additive is present mostly comprise significant π - π and dipole-dipole
472 interactions (e and s terms) along with strong interactions with cationic species (d^+
473 term), which may issue from the most electronegative fluorine atoms. With the
474 introduction of ammonium acetate and water, the most significant changes are:
475 reduction of dipole-dipole interactions (s term) and interactions with cations (d^+ term),
476 strong increase in dispersive interactions (v term) and interactions with anions (d'
477 term).

478

479 Finally, the last three columns are all polar stationary phases and will be discussed
480 as a group, as most observations are similar between the three phases. First, the
481 retention patterns are all consistent with “normal-phase” retention mode. Indeed, all
482 polar interaction terms (e , s , a , b , d and d^+) are positive, while the dispersive
483 interactions are negative (v term). This is similar to what is normally observed in
484 normal-phase HPLC [35,40]. When no additive is present, the most significant
485 interaction terms are hydrogen bonding interactions (a and b terms) with the silica
486 surface or polar ligands. Interactions with anionic species are rather small (d term)
487 while interactions with cationic species are positive (d^+ term). When the additives are
488 present in the mobile phase: dipole-dipole interactions and hydrogen bonding with
489 proton donors (s and a terms) increase on all three columns. Regarding ionic
490 interactions, as was observed in the previous section on the BEH silica phase, the
491 retention of cationic species slightly increases (d^+ term) while the retention of anionic
492 species strongly increases (d term). Clearly, the three polar phases seem most
493 significantly affected by the adsorption of additives than the less polar phases. This
494 was not unexpected as the adsorption of water and ammonium acetate should be
495 favoured on polar surfaces and ligands. This is in accordance with previous reports
496 from Berger and Deye, indicating that “polar columns exhibit as much as a 50-fold
497 higher additive surface coverage than non-polar columns” [23]. Similarly, the
498 adsorption of the methanol co-solvent was shown to vary depending on the polarity
499 of the stationary phase, in the works of Glenne et al. [25].

500

501 To gain a more global view of the different retention and separation behaviours in
502 such a wide variety of stationary phases, the spider diagram was previously designed
503 [30,35,41]. Very simply, it allows projecting the 7-dimensional space into 2
504 dimensions, with a minimal loss of information (Figure 5). Bubble points represent
505 stationary phases. The closer the position of the bubbles, the closer the retention
506 behaviour. Conversely, bubbles that are distant in the figure indicate different
507 retention behaviour, usually yielding different elution orders and selectivities. Bubble
508 size is related to the strength of interactions. Comparing the plots of stationary phase
509 classification with (Figure 5a) or without (Figure 5b) additives, an immediate
510 observation is that the bubbles representing the stationary phases are more
511 scattered in the selectivity space when no additive is present. On the opposite, when

512 ammonium acetate and water are present, many bubbles are grouped in close
513 fashion. This is especially true to the polar stationary phases on the right hand-side
514 of the figure. For instance, the polar stationary phases with a diversity of bonding
515 chemistries that are significantly different when no additive is present (although all
516 providing “normal-phase” retention mode) are all clustered together when additives
517 are present. Also the pentafluorophenyl phases (in the upper part of the figure) that
518 are most singular without additives (mostly due to strong interactions with cations)
519 are not so strongly different from the others when additives are present. This
520 indicates that the introduction of an additive is reducing the differences between the
521 stationary phases, probably because they are now all covered with a layer of water
522 molecules, ammonium and acetate ions.

523 A side-conclusion of this observation is that, while the additive is favourable to elution
524 of ionizable species (especially bases), it is not necessarily favourable to take
525 advantage of the selectivity differences of the stationary phases. Whenever the
526 analytes do not require the use of an additive to elute from the columns, it would then
527 be advisable to screen for stationary phases without it.

528
529 As all experiments in this paper were conducted at a rather low temperature (25°C),
530 which should definitely be in subcritical conditions and not subcritical, the applicability
531 of the above observations to higher temperatures is questionable. Blackwell and co-
532 workers had addressed that question previously [6] and concluded that higher
533 temperatures (80°C) favoured stronger interactions between analyte and stationary
534 phase, thereby favouring selectivity, and that the effects of additives were more
535 pronounced than was the case at lower temperature values (25°C). A possible
536 reason for these observations is that increasing temperature causes decreasing
537 adsorption of mobile phase components on the stationary phase. Then the stationary
538 phase surface and/or ligands are more available for analyte adsorption. Because the
539 stationary phase surface would be less shielded with mobile phase components,
540 different stationary phases would likely retain more singularity.

541

542 ***4.3. Effect of a salt additive in the sample dilution solvent***

543

544 Judging from the above observations (long equilibration times, loss of specific
545 selectivity) and others not further discussed here (possible damage to the stationary

546 phase, difficulty to clean the stationary phase from adsorbed additives), it would be
547 desirable to avoid their use whenever possible. However, for solubility reasons, some
548 analytes (very polar and/or ionizable species) do require the presence of an additive.
549 Rather than introducing it in the mobile phase composition, it was sometimes
550 recommended to introduce it solely in the dilution solvent for the analyte [18,19]. In
551 this experiment, again only the ACQUITY UPC² BEH column was examined with a
552 single basic analyte, nicotine. The analyte was diluted in methanol containing 5 mM
553 ammonium acetate and 0.5% water.

554 In Figure 6a, selected analyses from 30 replicates (injections 1, 5, 10, 15, 20, 25 and
555 30) can be observed. It is clear that the retention and peak shapes both take several
556 injections before to reach an equilibrium: retention time progressively reduces, with
557 peak symmetry progressively improving. A non-variant chromatogram was obtained
558 after about 25 injections. When increasing the concentration of additive in the dilution
559 solvent to 10 and 20 mM, peak shape was slightly improved and the number of
560 injections required to reach a reproducible chromatogram reduced to about 20.

561 Similar improvement in peak shape when concentration increased was previously
562 mentioned by Desfontaine *et al.* [19] but no mention was made on reproducibility.
563 Another interesting observation is to compare the best chromatogram obtained with
564 additives in dilution solvent to that obtained without additives and with the additives in
565 mobile phase (Figure 6b). While the introduction of additive in the dilution solvent is
566 yielding some improvement compared to no additive (retention decrease and slightly
567 improved peak shape), it is by no means as significant as the results obtained when
568 the additive is in the mobile phase. Highest concentrations yielded identical
569 conclusions. If the sole effect of additives in the dilution solvent had been to create
570 ion pairs with the analyte, the chromatograms should have been as good as the one
571 obtained when the additives are in the mobile phase, and from the first injection.
572 Most probably, the additives present in the dilution solvent get progressively
573 adsorbed on the stationary phase, until a stable layer is obtained and no further
574 changes are observed. It is possible however that less polar stationary phases would
575 have yielded more favourable conclusions, as less adsorption of additives should
576 occur. In another paper from Desfontaine *et al.* concerning the choice of dilution
577 solvent [42], it was concluded that the best solvent should be one with the lowest
578 adsorption possible on the stationary phase. This conclusion may also apply to
579 additives.

580 As a conclusion, based on the limited experiments above (one stationary phase, one
581 analyte, one additive), the sole use of an additive in the dilution solvent seems clearly
582 not advisable as (i) the results are clearly inferior to those obtained when additives
583 are present in the mobile phase and (ii) repeatability is very poor, as it takes many
584 injections to obtain repeatable analyses.

585

586

587 **5. Conclusions**

588 In the first part of this series, the limited effects of additives on mobile phase polarity,
589 but significant effects on the apparent acidity of the mobile phase had been

590 demonstrated. In this second instalment, the adsorption of ammonium acetate and
591 water on the stationary phases has been thoroughly investigated. On the one hand,
592 adsorption of mobile phase additives cause significant problems: long equilibration
593 time; levelling of retention patterns and selectivities, especially for polar stationary
594 phases, and poor reproducibility when small concentrations of additives are included

595 in the sample diluent. On another hand, the introduction of additives in the mobile
596 phase is mostly found to be beneficial to chromatographic resolution and detection.

597 The effects of ammonium acetate on the interactions established with a variety of
598 stationary phases have been deconvoluted with the help of LSER methodology.

599 Perhaps in contradiction to the usual impression, the retention of basic analytes is
600 not necessarily reduced by the introduction of ammonium acetate, although peak
601 shapes are generally improved. In addition, the retention of acidic analytes increases
602 significantly when the concentration of ammonium acetate increases. When the
603 stationary phases are covered with an additive, they are much more similar than
604 without an additive, especially polar stationary phases that adsorb more polar
605 additives. In the last section of this series of papers, we shall examine the possible
606 effects occurring between the analytes and additives.

607

608

609 **Acknowledgment**

610 We wish to thank the manufacturers who kindly provided the stationary phases for
611 this study: H el ene Boiteux, Marleen van Wingerden, Kevin Wyndham, Darryl
612 Brousmiche, Jacob Fairchild and Steve Collier (Waters), Thierry Domenger

613 (Thermo), Magali Dupin, Bitá Bordbar, Marc Jacob and Tivadar Farkas
614 (Phenomenex), Regis Guyon (Machery-Nagel), Mélanie Durand (Supelco), Mark
615 Woodruff (Fortis), and Akira Nakazawa (Nacalai). We also thank Waters for the
616 support received through the Centers of Innovation (COI). CW also thanks the Institut
617 Universitaire de France (IUF), of which she is a junior member, for the support
618 received.

619

620

621 **References**

622

- 623 [1] E. Lesellier, C. West, The many faces of packed column supercritical fluid
624 chromatography - A critical review, *J. Chromatogr. A.* 1382 (2015) 2–46.
625 doi:10.1016/j.chroma.2014.12.083.
- 626 [2] L. Nováková, A. Grand-Guillaume Perrenoud, I. Francois, C. West, E. Lesellier,
627 D. Guillarme, Modern analytical supercritical fluid chromatography using
628 columns packed with sub-2 μm particles: A tutorial, *Anal. Chim. Acta.* 824 (2014)
629 18–35. doi:10.1016/j.aca.2014.03.034.
- 630 [3] C. West, Current trends in supercritical fluid chromatography, *Anal. Bioanal.*
631 *Chem.* 410 (2018) 6441–6457. doi:10.1007/s00216-018-1267-4.
- 632 [4] K. Taguchi, E. Fukusaki, T. Bamba, Simultaneous analysis for water- and fat-
633 soluble vitamins by a novel single chromatography technique unifying
634 supercritical fluid chromatography and liquid chromatography, *J. Chromatogr. A.*
635 1362 (2014) 270–277. doi:10.1016/j.chroma.2014.08.003.
- 636 [5] V. Desfontaine, G.L. Losacco, Y. Gagnebin, J. Pezzatti, W.P. Farrell, V.
637 González-Ruiz, S. Rudaz, J.-L. Veuthey, D. Guillarme, Applicability of
638 supercritical fluid chromatography – mass spectrometry to metabolomics. I –
639 Optimization of separation conditions for the simultaneous analysis of
640 hydrophilic and lipophilic substances, *J. Chromatogr. A.* 1562 (2018) 96–107.
641 doi:10.1016/j.chroma.2018.05.055.
- 642 [6] J.A. Blackwell, R.W. Stringham, J.D. Weckwerth, Effect of Mobile Phase
643 Additives in Packed-Column Subcritical and Supercritical Fluid Chromatography,
644 *Anal. Chem.* 69 (1997) 409–415. doi:10.1021/ac9608883.
- 645 [7] K.W. Phinney, L.C. Sander, Additive concentration effects on enantioselective
646 separations in supercritical fluid chromatography, *Chirality.* 15 (2003) 287–294.
647 doi:10.1002/chir.10196.
- 648 [8] J.A. Blackwell, Effect of acidic mobile phase additives on chiral selectivity for
649 phenylalanine analogs using subcritical fluid chromatography, *Chirality.* 11
650 (1999) 91–97. doi:10.1002/(SICI)1520-636X(1999)11:2<91::AID-
651 CHIR2>3.0.CO;2-N.
- 652 [9] T.A. Berger, J.F. Deye, Separation of Benzene Polycarboxylic Acids by Packed
653 Column Supercritical Fluid Chromatography using Methanol-Carbon Dioxide

- 654 Mixtures with Very Polar Additives, *J. Chromatogr. Sci.* 29 (1991) 141–146.
655 doi:10.1093/chromsci/29.4.141.
- 656 [10] J.L. Janicot, M. Caude, R. Rosset, Separation of opium alkaloids by carbon
657 dioxide sub- and supercritical fluid chromatography with packed columns□:
658 Application to the quantitative analysis of poppy straw extracts, *J. Chromatogr.*
659 *A.* 437 (1988) 351–364. doi:10.1016/S0021-9673(00)90409-6.
- 660 [11] T.A. Berger, J.F. Deye, Effect of Basic Additives on Peak Shapes of Strong
661 Bases Separated by Packed-Column Supercritical Fluid Chromatography, *J.*
662 *Chromatogr. Sci.* 29 (1991) 310–317. doi:10.1093/chromsci/29.7.310.
- 663 [12] O. Gyllenhaal, J. Vessman, Packed-column supercritical fluid chromatography of
664 omeprazole and related compounds: Selection of column support with
665 triethylamine- and methanol-modified carbon dioxide as the mobile phase, *J.*
666 *Chromatogr. A.* 628 (1993) 275–281. doi:10.1016/0021-9673(93)80011-V.
- 667 [13] O. Gyllenhaal, J. Vessman, Potential of packed column supercritical fluid
668 chromatography for the separation of metoprolol from closely related
669 compounds, *J. Chromatogr. A.* 839 (1999) 141–148. doi:10.1016/S0021-
670 9673(99)00052-7.
- 671 [14] K. De Klerck, D. Mangelings, D. Clicq, F. De Boever, Y. Vander Heyden,
672 Combined use of isopropylamine and trifluoroacetic acid in methanol-containing
673 mobile phases for chiral supercritical fluid chromatography, *J. Chromatogr. A.*
674 1234 (2012) 72–79. doi:10.1016/j.chroma.2011.11.023.
- 675 [15] L.J. Mulcahey, L.T. Taylor, Gradient separation of pharmaceuticals employing
676 supercritical CO₂ and modifiers, *J. High Resolut. Chromatogr.* 13 (1990) 393–
677 396. doi:10.1002/jhrc.1240130602.
- 678 [16] D. Parlier, D. Thiébaud, M. Caude, R. Rosset, Supercritical fluid chromatography
679 of imidazole derivatives, *Chromatographia.* 31 (1991) 293–296.
680 doi:10.1007/BF02275753.
- 681 [17] V. Camel, D. Thiébaud, M. Caude, M. Dreux, Packed column subcritical fluid
682 chromatography of underivatized amino acids, *J. Chromatogr. A.* 605 (1992) 95–
683 101. doi:10.1016/0021-9673(92)85032-O.
- 684 [18] A. Cazenave-Gassiot, R. Boughtflower, J. Caldwell, L. Hitzel, C. Holyoak, S.
685 Lane, P. Oakley, F. Pullen, S. Richardson, G.J. Langley, Effect of increasing
686 concentration of ammonium acetate as an additive in supercritical fluid

- 687 chromatography using CO₂–methanol mobile phase, *J. Chromatogr. A.* 1216
688 (2009) 6441–6450. doi:10.1016/j.chroma.2009.07.022.
- 689 [19] V. Desfontaine, J.-L. Veuthey, D. Guillarme, Evaluation of innovative stationary
690 phase ligand chemistries and analytical conditions for the analysis of basic drugs
691 by supercritical fluid chromatography, *J. Chromatogr. A.* 1438 (2016) 244–253.
692 doi:10.1016/j.chroma.2016.02.029.
- 693 [20] C. West, J. Melin, H. Ansouri, M. Mengue Metogo, Unravelling the effects of
694 mobile phase additives in supercritical fluid chromatography. Part I: Polarity and
695 acidity of the mobile phase, *J. Chromatogr. A.* 1492 (2017) 136–143.
696 doi:10.1016/j.chroma.2017.02.066.
- 697 [21] J.R. Strubinger, H. Song, J.F. Parcher, High-pressure phase distribution
698 isotherms for supercritical fluid chromatographic systems. 1. Pure carbon
699 dioxide, *Anal. Chem.* 63 (1991) 98–103. doi:10.1021/ac00002a003.
- 700 [22] J.R. Strubinger, H. Song, J.F. Parcher, High-pressure phase distribution
701 isotherms for supercritical fluid chromatographic systems. 2. Binary isotherms of
702 carbon dioxide and methanol, *Anal. Chem.* 63 (1991) 104–108.
703 doi:10.1021/ac00002a004.
- 704 [23] T.A. Berger, J.F. Deye, Role of additives in packed column supercritical fluid
705 chromatography: suppression of solute ionization, *J. Chromatogr. A.* 547 (1991)
706 377–392. doi:10.1016/S0021-9673(01)88661-1.
- 707 [24] A. Rajendran, Local equilibrium theory analysis of chromatographic peak shapes
708 in the presence of adsorbing modifiers, *J. Chromatogr. A.* 1485 (2017) 52–61.
709 doi:10.1016/j.chroma.2017.01.009.
- 710 [25] E. Glenne, K. Öhlén, H. Leek, M. Klarqvist, J. Samuelsson, T. Fornstedt, A
711 closer study of methanol adsorption and its impact on solute retentions in
712 supercritical fluid chromatography, *J. Chromatogr. A.* 1442 (2016) 129–139.
713 doi:10.1016/j.chroma.2016.03.006.
- 714 [26] E. Glenne, H. Leek, M. Klarqvist, J. Samuelsson, T. Fornstedt, Peak
715 deformations in preparative supercritical fluid chromatography due to co-solvent
716 adsorption, *J. Chromatogr. A.* 1468 (2016) 200–208.
717 doi:10.1016/j.chroma.2016.09.019.
- 718 [27] E. Glenne, H. Leek, M. Klarqvist, J. Samuelsson, T. Fornstedt, Systematic
719 investigations of peak deformations due to co-solvent adsorption in preparative

- 720 supercritical fluid chromatography, *J. Chromatogr. A.* 1496 (2017) 141–149.
721 doi:10.1016/j.chroma.2017.03.053.
- 722 [28] D. Speybrouck, C. Doublet, P. Cardinael, C. Fiol-Petit, D. Corens, The effect of
723 high concentration additive on chiral separations in supercritical fluid
724 chromatography, *J. Chromatogr. A.* 1510 (2017) 89–99.
725 doi:10.1016/j.chroma.2017.06.049.
- 726 [29] E. Lemasson, S. Bertin, P. Hennig, H. Boiteux, E. Lesellier, C. West,
727 Development of an achiral supercritical fluid chromatography method with
728 ultraviolet absorbance and mass spectrometric detection for impurity profiling of
729 drug candidates. Part I: Optimization of mobile phase composition, *J.*
730 *Chromatogr. A.* 1408 (2015) 217–226. doi:10.1016/j.chroma.2015.07.037.
- 731 [30] C. West, E. Lemasson, S. Bertin, P. Hennig, E. Lesellier, An improved
732 classification of stationary phases for ultra-high performance supercritical fluid
733 chromatography, *J. Chromatogr. A.* 1440 (2016) 212–228.
734 doi:10.1016/j.chroma.2016.02.052.
- 735 [31] M. Vitha, P.W. Carr, The chemical interpretation and practice of linear solvation
736 energy relationships in chromatography, *J. Chromatogr. A.* 1126 (2006) 143–
737 194. doi:10.1016/j.chroma.2006.06.074.
- 738 [32] R.-I. Chirita, C. West, S. Zubrzycki, A.-L. Finaru, C. Elfakir, Investigations on the
739 chromatographic behaviour of zwitterionic stationary phases used in hydrophilic
740 interaction chromatography, *Hydrophilic Interact. Chromatogr.* 1218 (2011)
741 5939–5963. doi:10.1016/j.chroma.2011.04.002.
- 742 [33] C. West, E. Lemasson, S. Khater, E. Lesellier, An attempt to estimate ionic
743 interactions with phenyl and pentafluorophenyl stationary phases in supercritical
744 fluid chromatography, *J. Chromatogr. A.* 1412 (2015) 126–138.
745 doi:10.1016/j.chroma.2015.08.009.
- 746 [34] W.P. Farrell, Practical Approaches to Column Selection for Supercritical Fluid
747 Chromatography, in: *Supercrit. Fluid Chromatogr.*, Elsevier, 2017: pp. 57–101.
748 doi:10.1016/B978-0-12-809207-1.00003-3.
- 749 [35] C. West, E. Lesellier, A unified classification of stationary phases for packed
750 column supercritical fluid chromatography, *J. Chromatogr. A.* 1191 (2008) 21–
751 39. doi:10.1016/j.chroma.2008.02.108.

- 752 [36] C. West, E. Auroux, Deconvoluting the effects of buffer salt concentration in
753 hydrophilic interaction chromatography on a zwitterionic stationary phase, *J.*
754 *Chromatogr. A.* 1461 (2016) 92–97. doi:10.1016/j.chroma.2016.07.059.
- 755 [37] C. West, E. Lesellier, Effects of mobile phase composition on retention and
756 selectivity in achiral supercritical fluid chromatography, *J. Chromatogr. A.* 1302
757 (2013) 152–162. doi:10.1016/j.chroma.2013.06.003.
- 758 [38] T.A. Berger, J.F. Deye, Separation of Hydroxybenzoic Acids by Packed Column
759 Supercritical Fluid Chromatography using Modified Fluids with Very Polar
760 Additives, *J. Chromatogr. Sci.* 29 (1991) 26–30. doi:10.1093/chromsci/29.1.26.
- 761 [39] C. West, E. Lesellier, Characterization of stationary phases in subcritical fluid
762 chromatography by the solvation parameter model, *J. Chromatogr. A.* 1110
763 (2006) 181–190. doi:10.1016/j.chroma.2006.01.125.
- 764 [40] C. West, E. Lesellier, Characterisation of stationary phases in subcritical fluid
765 chromatography with the solvation parameter model, *J. Chromatogr. A.* 1110
766 (2006) 200–213. doi:10.1016/j.chroma.2006.01.109.
- 767 [41] C. West, E. Lesellier, Characterisation of stationary phases in subcritical fluid
768 chromatography by the solvation parameter model, *J. Chromatogr. A.* 1110
769 (2006) 191–199. doi:10.1016/j.chroma.2006.02.002.
- 770 [42] V. Desfontaine, A. Tarafder, J. Hill, J. Fairchild, A. Grand-Guillaume Perrenoud,
771 J.-L. Veuthey, D. Guillarme, A systematic investigation of sample diluents in
772 modern supercritical fluid chromatography, *J. Chromatogr. A.* 1511 (2017) 122–
773 131. doi:10.1016/j.chroma.2017.06.075.
- 774
- 775

776 **Figure Caption**

777

778 **Figure 1.** Breakthrough curves recorded with a step gradient from 0 to 5% co-solvent
779 in CO₂. Acquity UPC² BEH column at 25°C, 150 bar, 1 ml/min. The black, flat curve
780 was recorded with methanol alone while the orange, irregular curve was recorded
781 with methanol comprising 2% water and 20 mM ammonium acetate.

782

783 **Figure 2.** Variation of retention (log *k* values) for three selected analytes when the
784 concentration of ammonium acetate in the methanol co-solvent increases from 0 to
785 25 mM. Acquity UPC² BEH column at 25°C, 150 bar, 1 ml/min. Mobile phase: carbon
786 dioxide – methanol, with or without additive.

787

788 **Figure 3.** System map (variation of LSER system constants, calculated with the
789 retention data for the analytes in Table S1) on Acquity UPC² BEH column when the
790 concentration of ammonium acetate in the methanol co-solvent increases from 0 to
791 25 mM. 25°C, 150 bar, 1 ml/min. Mobile phase: carbon dioxide – methanol, with or
792 without additive. (a) System constants related to charge-neutral species and (b)
793 system constants for ionizable species.

794

795 **Figure 4.** Representative histograms for normalized LSER models calculated with
796 the retention data measured for the 114 analytes in Table 1 and Eq. (2).
797 Chromatographic conditions: CO₂-solvent 90:10 (v/v), 25°C, 150 bar, 1 or 3 mL/min
798 depending on column dimensions. The solvent is methanol alone (left, blue bars) or
799 containing 20 mM ammonium acetate and 2% water (right, orange bars).

800

801 **Figure 5.** Spider diagram based on LSER models calculated with Eq. (2), with the
802 retention data measured for the analytes in Table S1, for the 32 columns in Table 1.
803 Columns groups were defined in previous works according to similarities in
804 selectivity. Chromatographic conditions: CO₂-MeOH 90:10 (v/v), 25°C, 150 bar, 1 or
805 3 mL/min depending on column dimensions. (a) no additives, 109 eluted analytes (b)
806 with 2% water and 20 mM ammonium acetate in the methanol co-solvent, 114 eluted
807 analytes.

808

809 **Figure 6.** Variation of retention and peak shape observed for a basic analyte
810 (nicotine) eluted from ACQUITY UPC² BEH column when additives are present in the
811 dilution solvent. 25°C, 150 bar, 1 ml/min. CO₂-MeOH 90:10 (v/v), with 5 mM AA and
812 0.5% water in injection solvent. (a) Variation over 30 replicates. The chromatograms
813 shown are injections 1, 5, 10, 15, 20, 25 and 30. (b) Comparison to other conditions
814 when no additive is present or additive is present in the mobile phase.
815
816

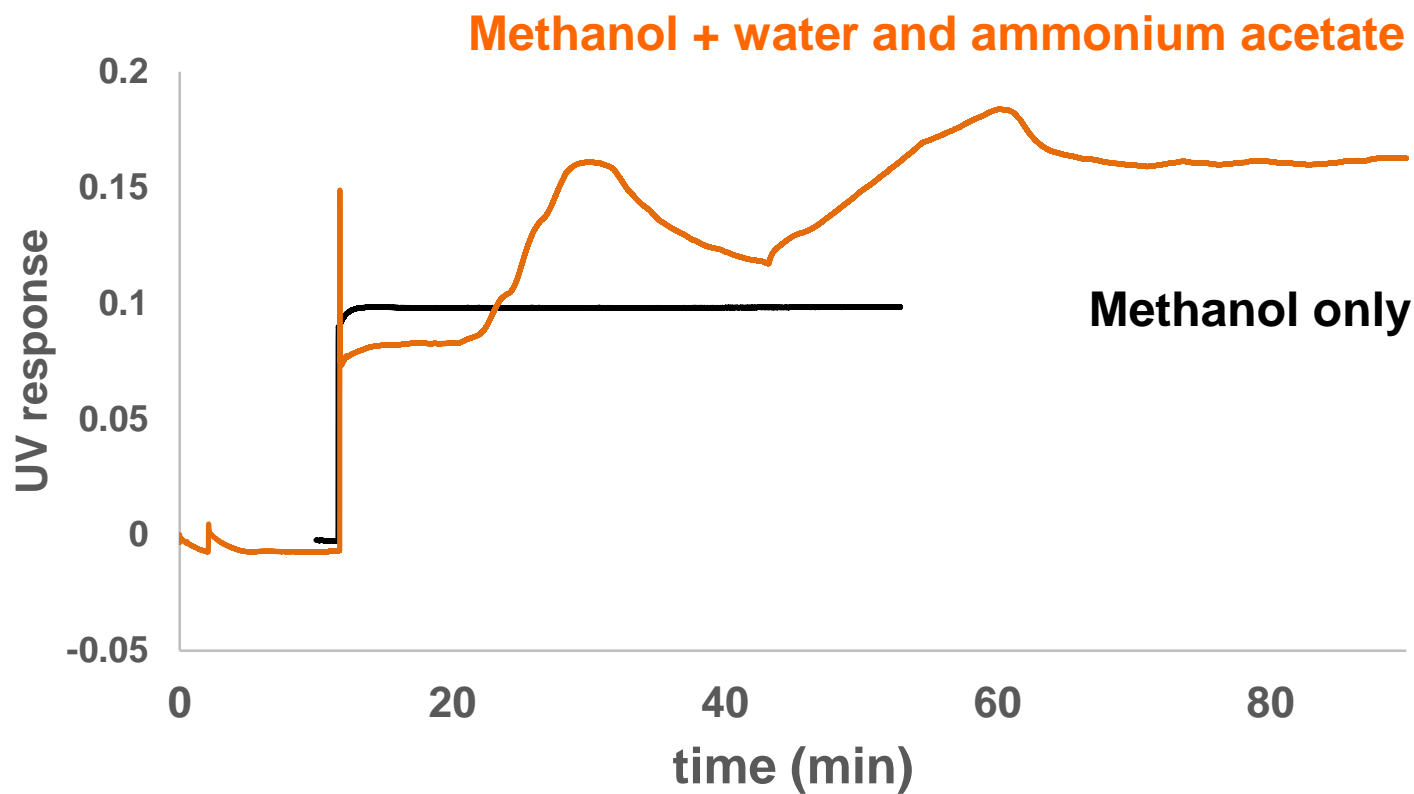


Figure 1. Breakthrough curves recorded with a step gradient from 0 to 5% co-solvent in CO₂. Acquity UPC² BEH column at 25°C, 150 bar, 1 ml/min. The black, flat curve was recorded with methanol alone while the orange, irregular curve was recorded with methanol comprising 2% water and 20 mM ammonium acetate.

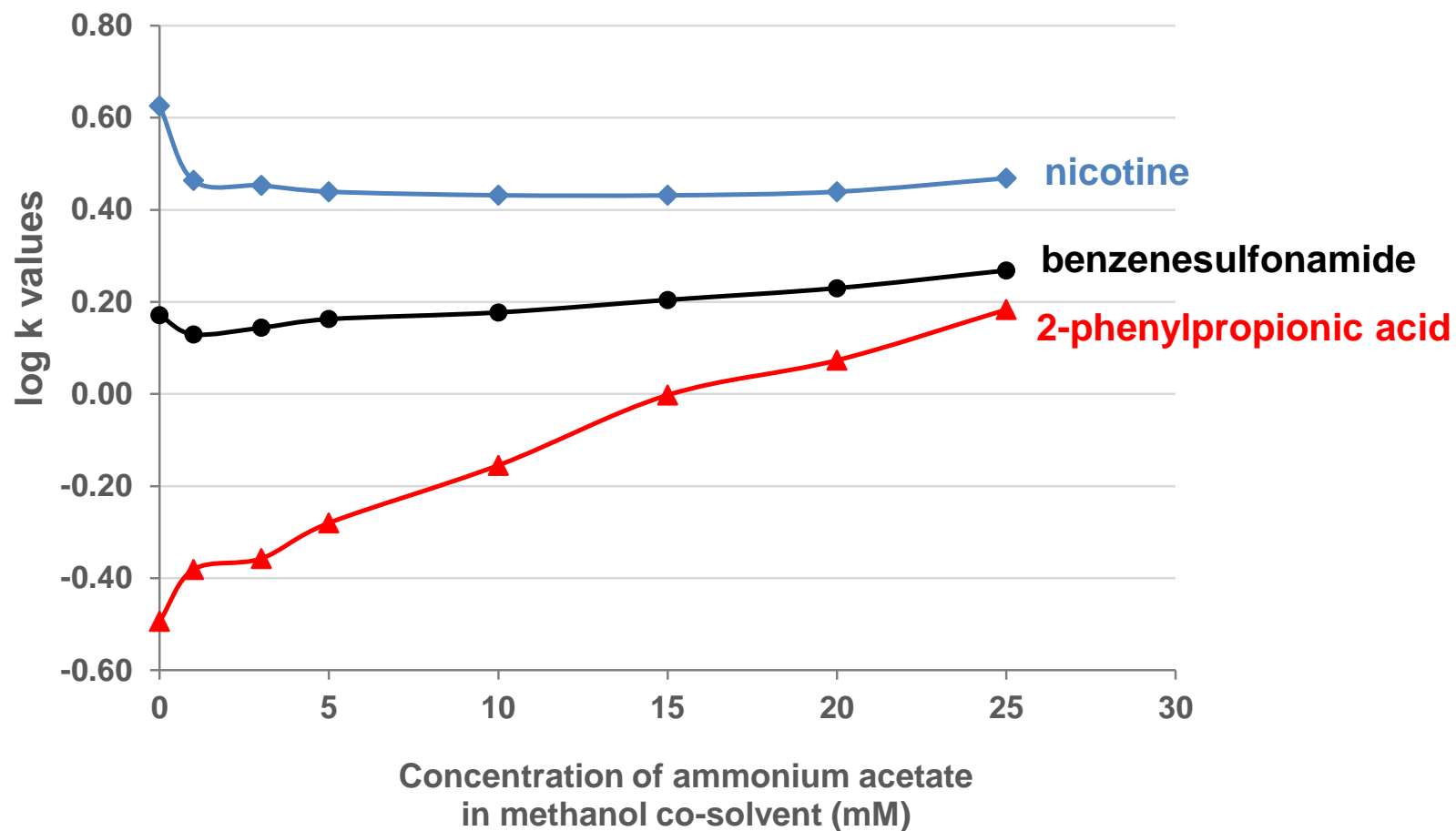


Figure 2. Variation of retention ($\log k$ values) for three selected analytes when the concentration of ammonium acetate in the methanol co-solvent increases from 0 to 25 mM. Acquity UPC² BEH column at 25°C, 150 bar, 1 ml/min. Mobile phase: carbon dioxide – methanol, with or without additive.

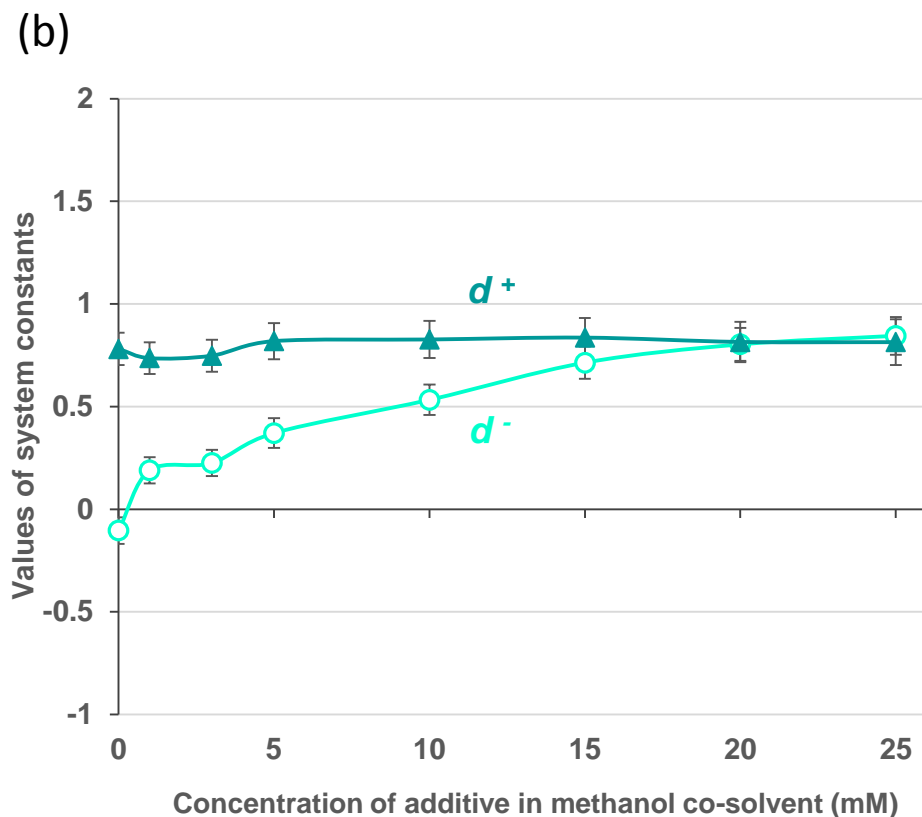
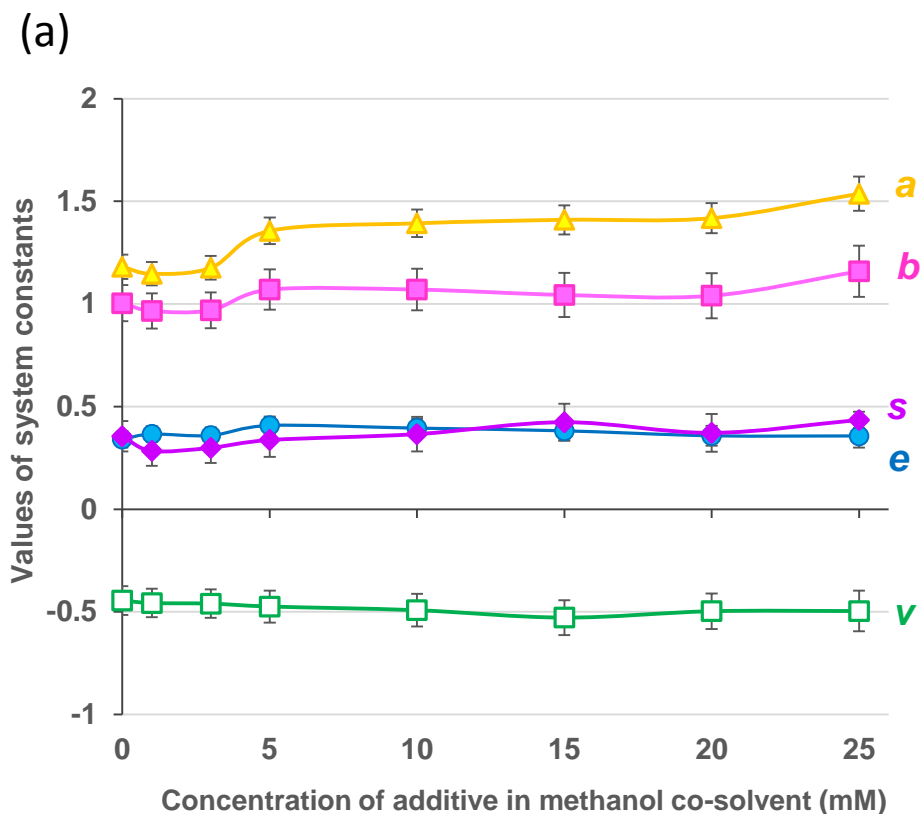
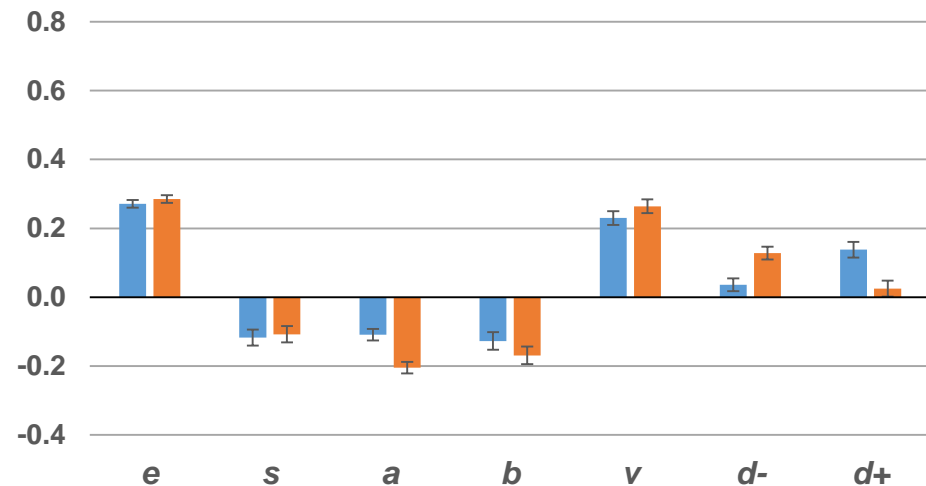
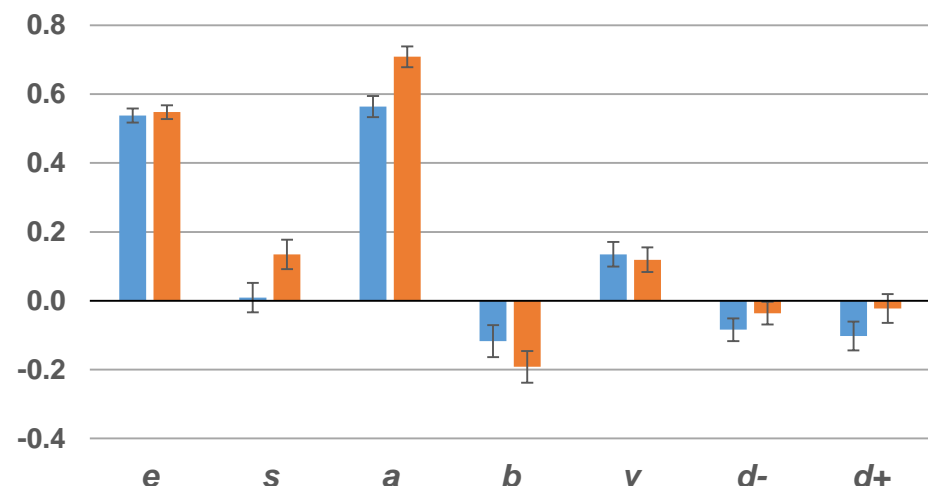


Figure 3. System map (variation of LSER system constants, calculated with the retention data for the analytes in Table S1) on Acquity UPC² BEH column when the concentration of ammonium acetate in the methanol co-solvent increases from 0 to 25 mM. 25°C, 150 bar, 1 ml/min. Mobile phase: carbon dioxide – methanol, with or without additive. (a) System constants related to charge-neutral species and (b) system constants for ionizable species.

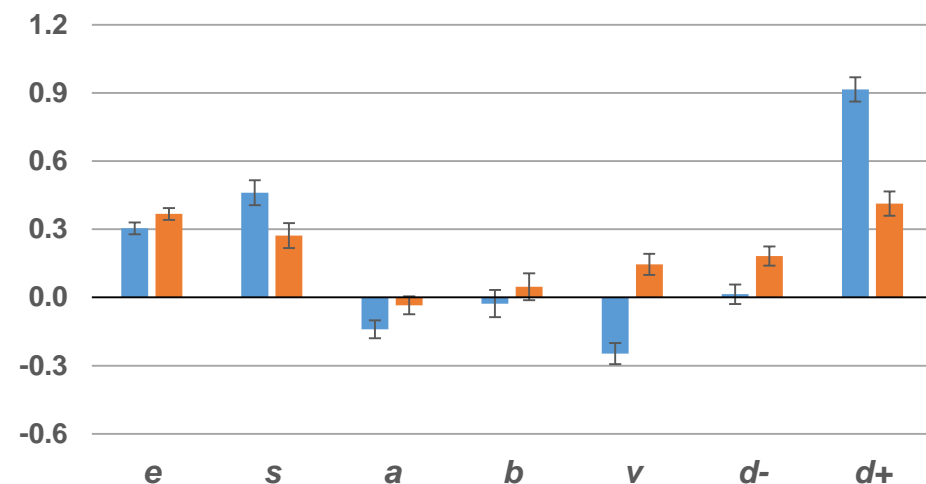
Accucore Phenyl-hexyl



Accucore Phenyl-X



Accucore PFP



Accucore HILIC

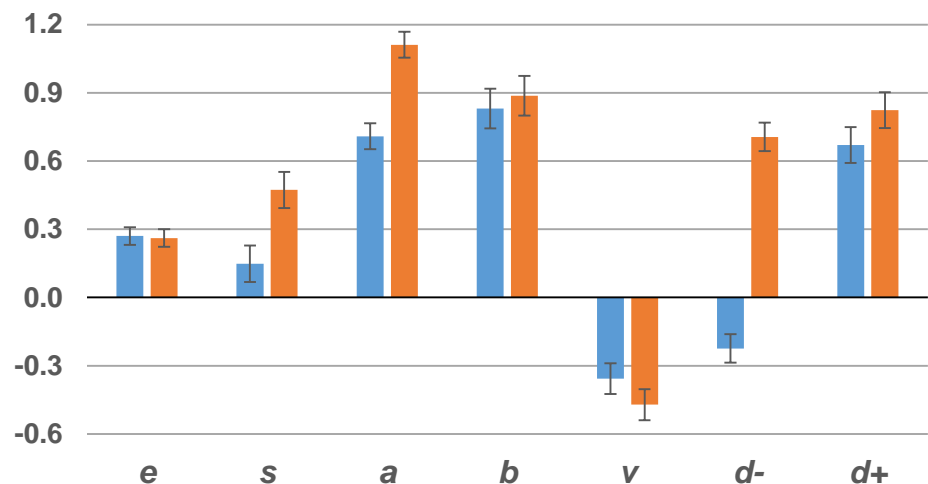
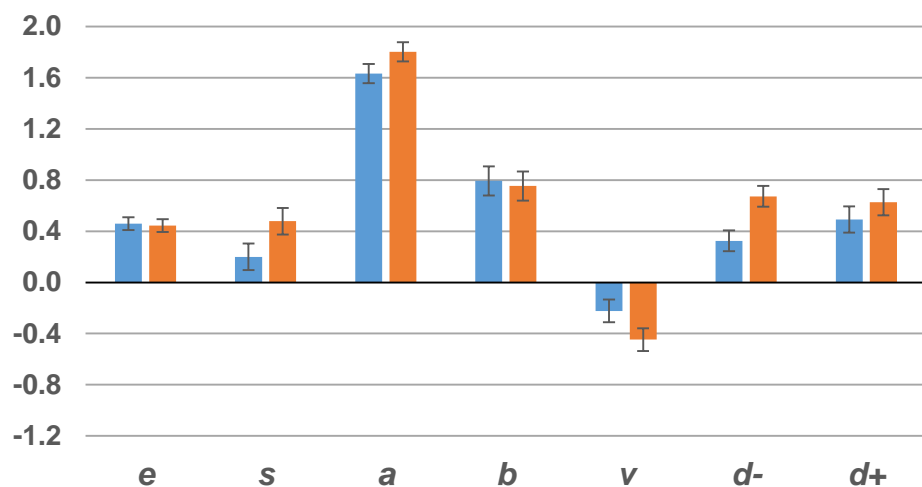


Figure 4. Representative histograms for normalized LSER models calculated with the retention data measured for the 114 analytes in Table 1 and Eq. (2). Chromatographic conditions: CO₂-solvent 90:10 (v/v), 25°C, 150 bar, 1 or 3 mL/min depending on column dimensions. The solvent is methanol alone (left, blue bars) or containing 20 mM ammonium acetate and 2% water (right, orange bars).

Accucore Urea



Accucore Amide

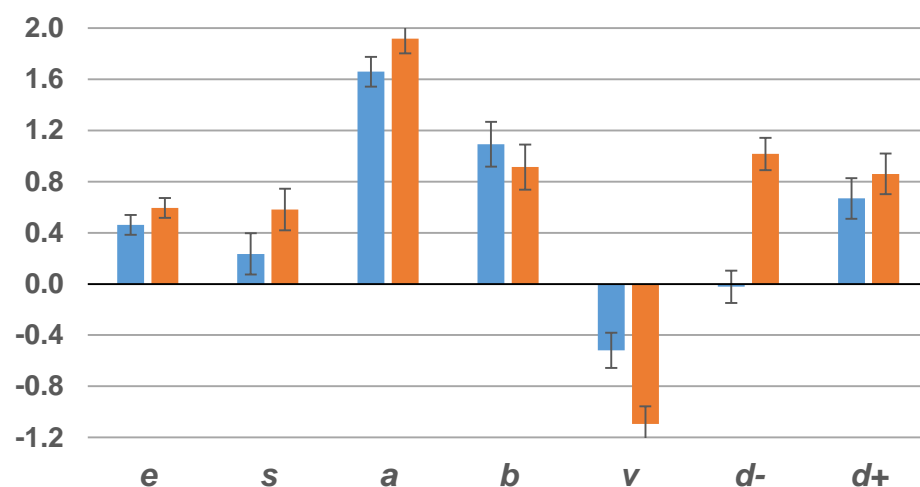


Figure 4 (continued). Representative histograms for normalized LSER models calculated with the retention data measured for the 114 analytes in Table 1 and Eq. (2). Chromatographic conditions: CO₂-solvent 90:10 (v/v), 25°C, 150 bar, 1 or 3 mL/min depending on column dimensions. The solvent is methanol alone (left, blue bars) or containing 20 mM ammonium acetate and 2% water (right, orange bars).

(a)

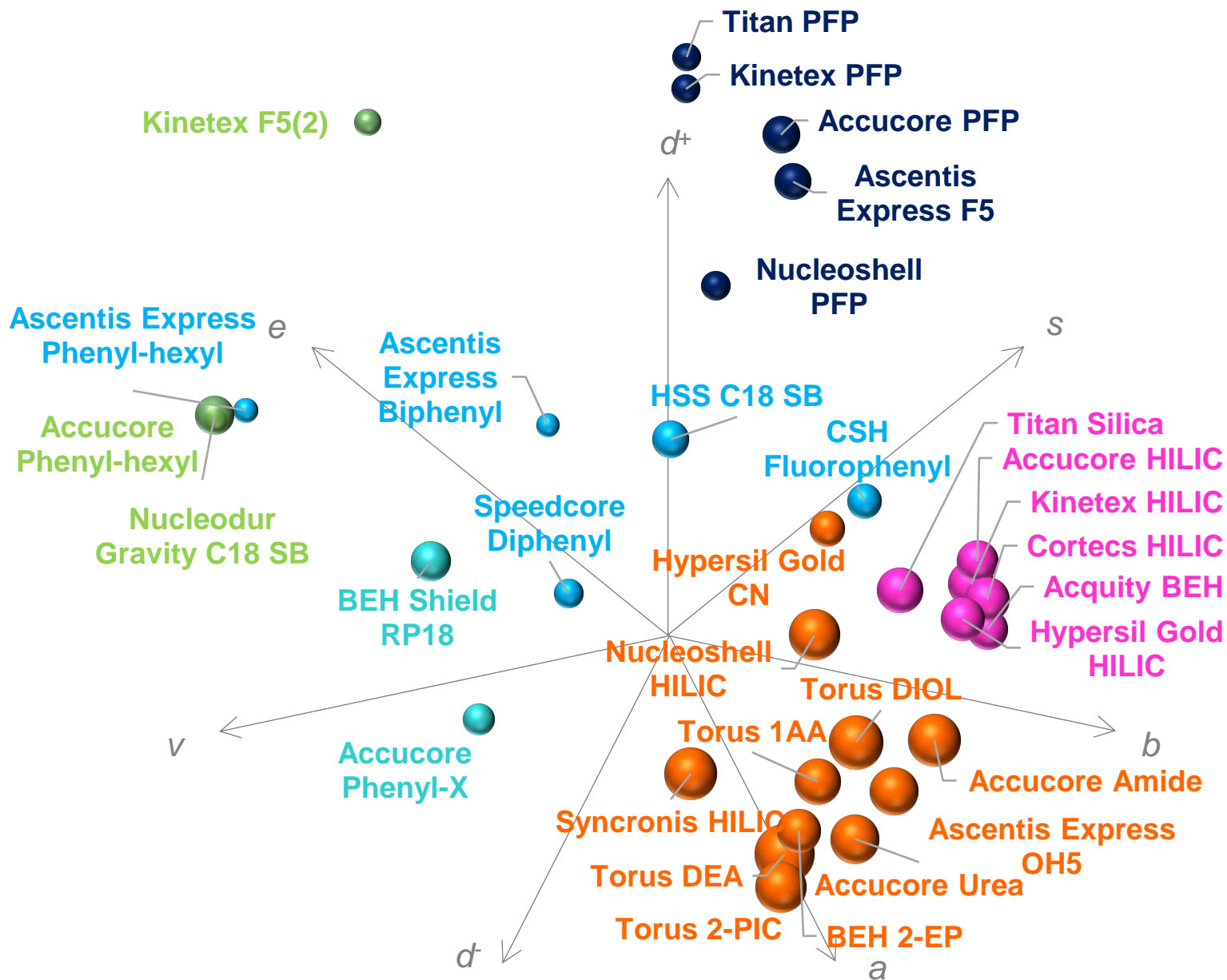


Figure 5. Spider diagram based on LSER models calculated with Eq. (2), with the retention data measured for the analytes in Table S1, for the 32 columns in Table 1. Columns groups were defined in previous works according to similarities in selectivity. Chromatographic conditions: CO₂-MeOH 90:10 (v/v), 25°C, 150 bar, 1 or 3 mL/min depending on column dimensions. (a) no additives, 109 eluted analytes (b) with 2% water and 20 mM ammonium acetate in the methanol co-solvent, 114 eluted analytes.

(b)

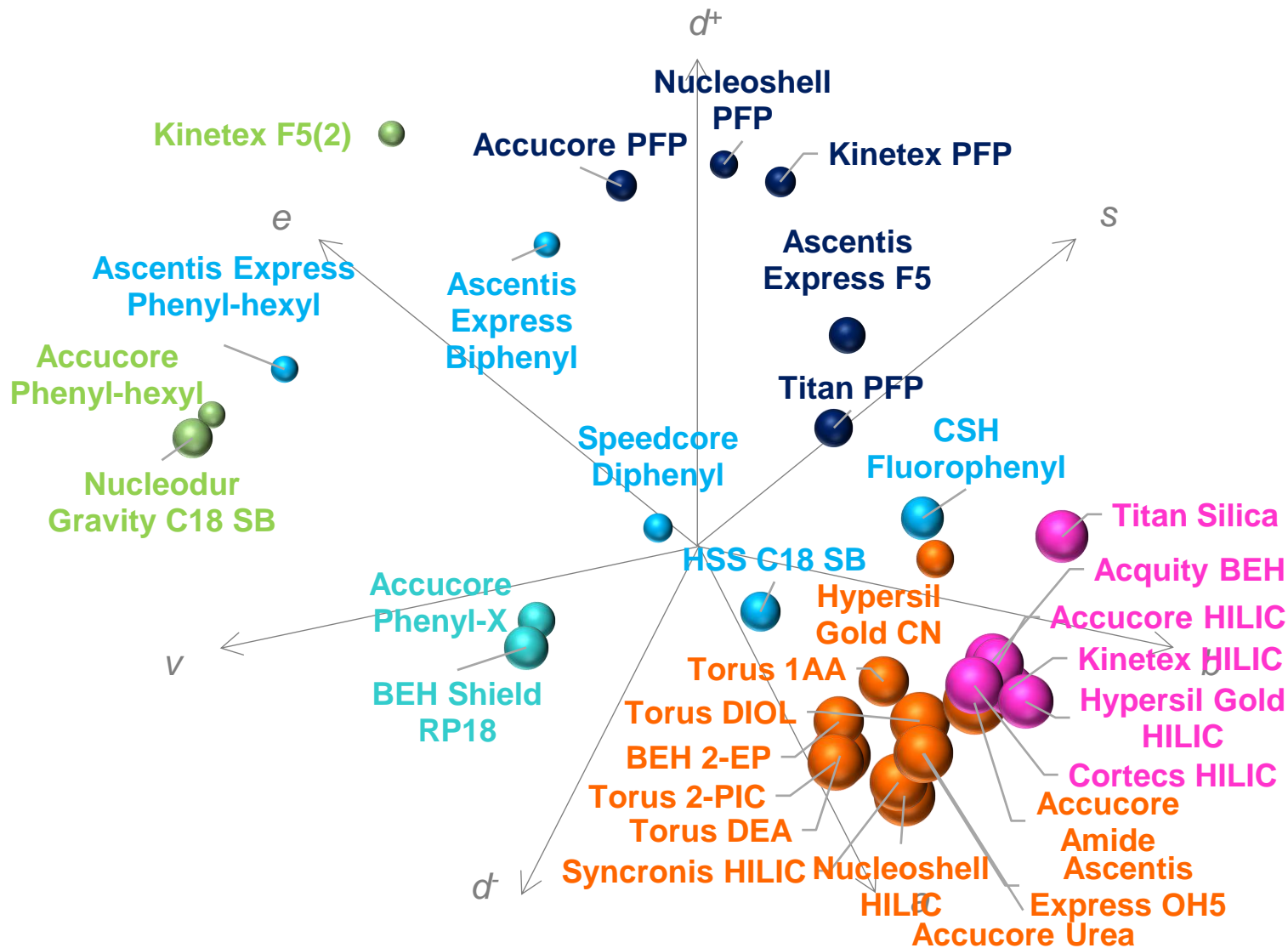


Figure 5. Spider diagram based on LSER models calculated with Eq. (2), with the retention data measured for the analytes in Table S1, for the 32 columns in Table 1. Columns groups were defined in previous works according to similarities in selectivity. Chromatographic conditions: CO_2 -MeOH 90:10 (v/v), 25°C, 150 bar, 1 or 3 mL/min depending on column dimensions. (a) no additives, 109 eluted analytes (b) with 2% water and 20 mM ammonium acetate in the methanol co-solvent, 114 eluted analytes.

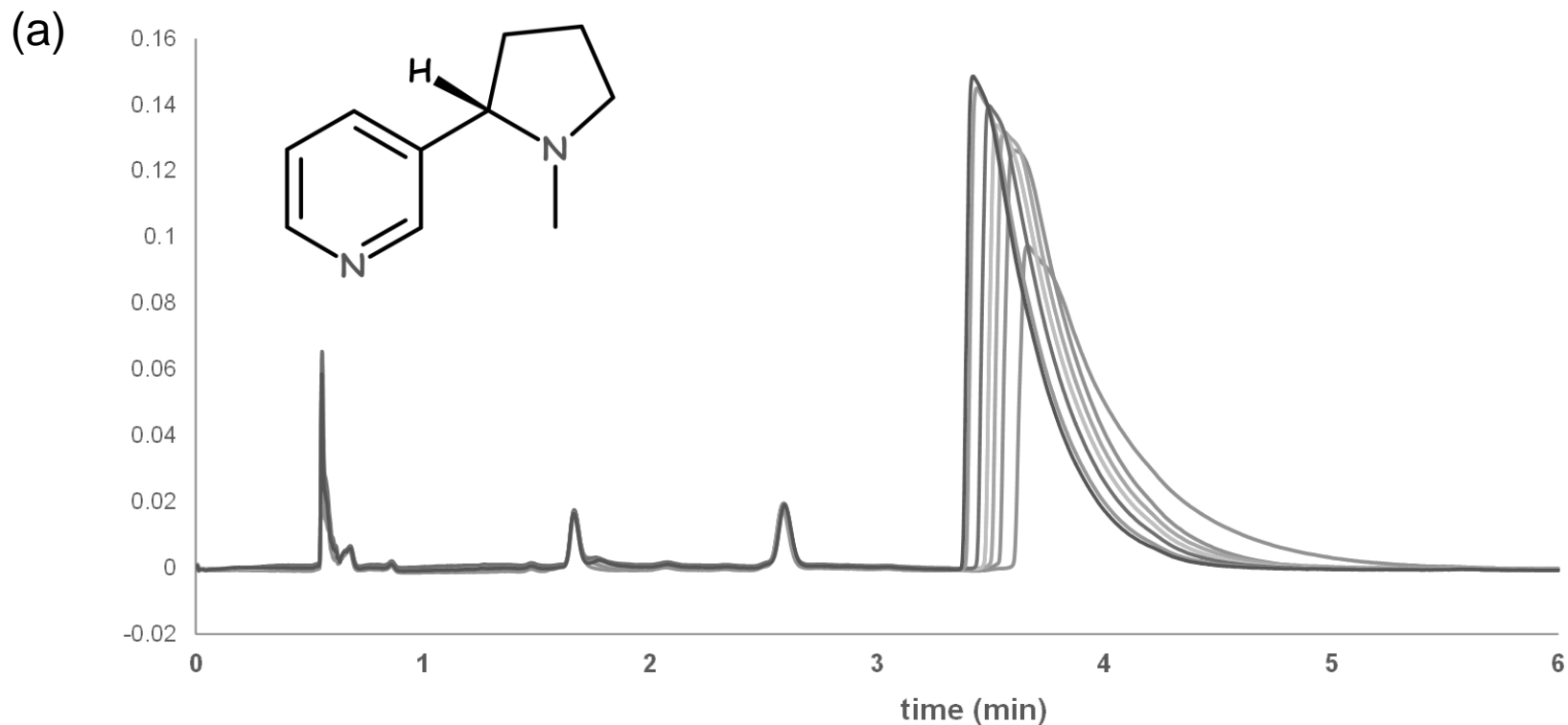


Figure 6. Variation of retention and peak shape observed for a basic analyte (nicotine) eluted from ACQUITY UPC² BEH column when additives are present in the dilution solvent. 25°C, 150 bar, 1 ml/min. CO₂-MeOH 90:10 (v/v), with 5 mM AA and 0.5% water in injection solvent. (a) Variation over 30 replicates. The chromatograms shown are injections 1, 5, 10, 15, 20, 25 and 30. (b) Comparison to other conditions when no additive is present or additive is present in the mobile phase.

(b)

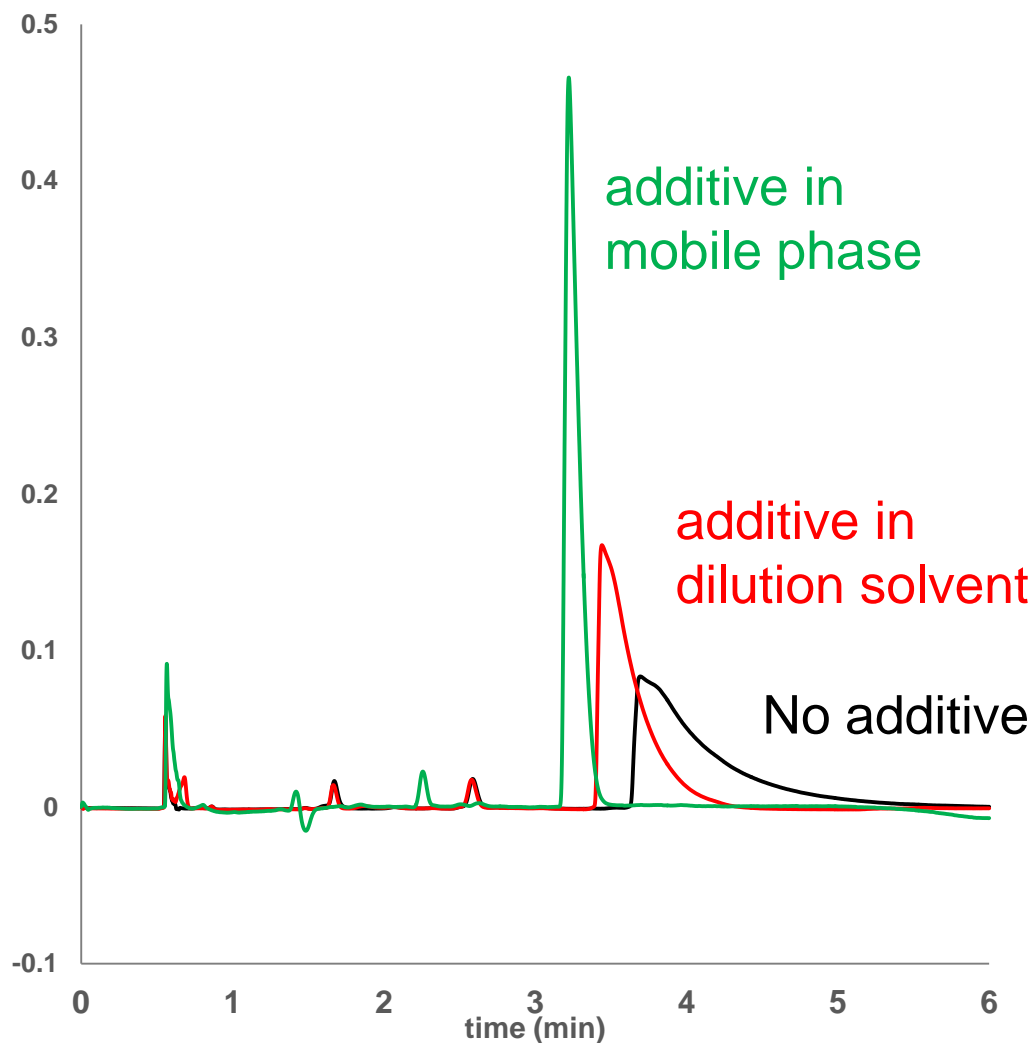


Figure 6. Variation of retention and peak shape observed for a basic analyte (nicotine) eluted from ACQUITY UPC² BEH column when additives are present in the dilution solvent. 25°C, 150 bar, 1 ml/min. CO₂-MeOH 90:10 (v/v), with 5 mM AA and 0.5% water in injection solvent. (a) Variation over 30 replicates. The chromatograms shown are injections 1, 5, 10, 15, 20, 25 and 30. (b) Comparison to other conditions when no additive is present or additive is present in the mobile phase (20 mM AA and 2 % water).

Table 1. 32 columns compared in the present study
 FPP stands for fully porous particles; SPP stands for superficially porous particles

Column Name	Manufacturer	Particles	Bonded ligand	Dimensions (mm)	Particle size (µm)
ACQUITY UPC ² HSS C18 SB	Waters	FPP	Octadecyl, non endcapped	100 x 3.0	1.8
ACQUITY UPC ² BEH	Waters	FPP	Bare hybrid silica	100 x 3.0	1.7
ACQUITY UPLC BEH Shield RP18	Waters	FPP	Alkyl with embedded carbamate group	100 x 3.0	1.7
ACQUITY UPC ² BEH 2-EP	Waters	FPP	2-ethylpyridine	100 x 3.0	1.7
ACQUITY UPC ² CSH Fluorophenyl	Waters	FPP	Pentafluorophenyl	100 x 3.0	1.7
ACQUITY UPC ² Torus 1-AA	Waters	FPP	1-Amino-anthracene	100 x 3.0	1.7
ACQUITY UPC ² Torus 2-PIC	Waters	FPP	2-Picolyl-amine	100 x 3.0	1.7
ACQUITY UPC ² Torus DEA	Waters	FPP	Diethylamine	100 x 3.0	1.7
ACQUITY UPC ² Torus DIOL	Waters	FPP	Propanediol	100 x 3.0	1.7
Nucleodur C18 Gravity-SB	Macherey-Nagel	FPP	Octadecyl, endcapped	100 x 3.0	1.8
Hypersil Gold Silica	Thermo	FPP	Bare silica	100 x 3.0	1.9
Hypersil Gold CN	Thermo	FPP	Cyanopropyl-bonded silica	100 x 2.1	1.9
Synchronis HILIC	Thermo	FPP	Sulfobetaine	100 x 3.0	1.7
Titan PFP	Sigma-Aldrich	FPP	Pentafluorophenyl	100 x 3.0	1.9
Titan Silica	Sigma-Aldrich	FPP	Bare silica	100 x 3.0	1.9
Accucore HILIC	Thermo	SPP	Bare silica	150 x 4.6	2.6
Accucore 150-Amide-HILIC	Thermo	SPP	Polyamide	150 x 4.6	2.6
Accucore Urea-HILIC	Thermo	SPP	Propylurea	150 x 4.6	2.6
Accucore Phenyl-X	Thermo	SPP	Phenyl-alkyl	150 x 4.6	2.6
Accucore Phenyl-hexyl	Thermo	SPP	Phenyl-hexyl	150 x 4.6	2.6
Accucore PFP	Thermo	SPP	Pentafluorophenyl	150 x 4.6	2.6
Ascentis Express Biphenyl	Sigma-Aldrich	SPP	Biphenyl	150 x 4.6	2.6
Ascentis Express Phenyl-hexyl	Sigma-Aldrich	SPP	Phenyl-hexyl	150 x 4.6	2.6
Ascentis Express OH5	Sigma-Aldrich	SPP	Penta-hydroxyl	150 x 4.6	2.7
Ascentis Express F5	Sigma-Aldrich	SPP	Pentafluorophenyl	150 x 4.6	2.7
Cortecs HILIC	Waters	SPP	Bare silica	150 x 4.6	2.7
Kinetex HILIC	Phenomenex	SPP	Bare silica	150 x 4.6	2.6
Kinetex PFP	Phenomenex	SPP	Pentafluorophenyl	150 x 4.6	2.6
Kinetex F5 (2)	Phenomenex	SPP	Pentafluorophenyl	150 x 4.6	2.6
Nucleoshell HILIC	Macherey-Nagel	SPP	Sulfobetaine	150 x 3.0	2.7
Nucleoshell PFP	Macherey-Nagel	SPP	Pentafluorophenyl	150 x 3.0	2.7
Speedcore Diphenyl	Fortis Technologies	SPP	Diphenyl-alkyl	150 x 4.6	2.6