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Characterization of retention and separation mechanisms with Pirkle-type enantioselective stationary phases in supercritical fluid chromatography

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► To cite this version:

Caroline West, Syame Khater. Characterization of retention and separation mechanisms with Pirkle-type enantioselective stationary phases in supercritical fluid chromatography. *Journal of Chromatography A*, 2020, 1626, pp.461352 -. 10.1016/j.chroma.2020.461352 . hal-03490206

HAL Id: hal-03490206

<https://hal.science/hal-03490206v1>

Submitted on 23 Jun 2022

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Abstract

In the present study, we characterize a famous Pirkle-type enantioselective stationary phase ((R,R)-Whelk-O1 from Regis Technologies) and an equivalent enantiomeric phase (ReproSil Chiral-NR from Dr. Maisch) in supercritical fluid chromatography (SFC) with carbon dioxide – methanol (90:10 v/v) mobile phase. First, the interactions contributing to retention are evaluated with a modified version of the solvation parameter model, comprising five Abraham descriptors (E, S, A, B, V), two additional descriptors to take account of molecular shape (flexibility F and globularity G), and two additional descriptors to take account of interactions with ionizable species (D^- and D^+). Linear solvation energy relationships (LSER) are established based on the retention of 212 achiral analytes. As expected, π - π interactions are the most significant to explain retention, while dipole-dipole, hydrogen bonding and ionic interactions with cationic species are of secondary importance.

Secondly, the contributions of interactions to enantioseparations are discussed, based on the analysis of 79 racemates. Discriminant analyses (DA) were computed to gain some insight on retention mechanisms. The set of racemates is first divided between racemates eluted earlier than expected based on the LSER models, and those eluted later than expected. Small spherical molecules are more retained than expected, as they may better fit inside the cleft of the chiral selector. They are also most frequently resolved, probably for the same reason. Among the molecules that are less retained than expected, which are rather large and/or non-spherical, other features are favourable to enantiorecognition: π -electrons, dipoles and electron-donating properties. Contrary to the observations on other sorts of chiral selectors, flexibility was found to have no contribution on the enantiorecognition process.

Keywords: chiral stationary phases; discriminant analysis; enantioseparation mechanisms; Pirkle-type phases; solvation parameter model; supercritical fluid chromatography.

50 1. Introduction

51
52 Brush-type chiral stationary phases (CSPs) [1] were among the first chiral selectors
53 purposely designed for liquid-phase chromatographic resolution of enantiomers. Previously,
54 naturally occurring chiral selectors like proteins [2] or amino acids (particularly for ligand-
55 exchange chromatography [3]) had been immobilized on silica supporting phases for use in
56 enantioselective liquid chromatography. Brush-type CSPs with charge-neutral synthetic
57 selectors were initially developed by William H. Pirkle and co-workers from the end of the
58 1970s [4,5], which is why these phases are often named “Pirkle-type”. The idea behind the
59 ligands proposed was to offer three points of interaction that would comply with the “three-
60 point attachment model” initially proposed by Easson and Stedman for protein binding [6],
61 and later adapted for enantioselective chromatography by Dalgliesh [7]. Although not entirely
62 accurate in describing the enantioseparation process, it is still convenient to design such
63 chiral selectors based on this model. As appears with the example in Figure 1, the chiral
64 ligands are designed to have three (or more) different interaction points. However, what does
65 not appear on this figure is that the bulky ligands also have a well-defined, rather rigid spatial
66 arrangement [8–10], with the tetrahydrophenanthrene ring and the dinitrobenzyl ring being
67 positioned in an orthogonal fashion, thereby forming a cleft. The dinitrobenzyl group appears
68 to be the only one with some conformational flexibility, somewhat rotating to bind the
69 analytes inside or outside the cleft [8,10]. As a result, chiral discrimination is favoured when
70 steric fit occurs, that is to say when the enantiomers have the adequate size, shape and
71 three-dimensional organization of the interacting groups to fit in the chiral cleft of the ligand
72 [5,11]. Three-dimensional structures have been shown in the past, based on crystallographic
73 measurements [11] or based on molecular modelling [8–10].
74 Compared to other CSPs that are now frequently used like polysaccharides [12–14],
75 macrocyclic glycopeptides [15–17] or protein-based phases [2], one advantage of such
76 brush-type phases is that the ligand has a controlled chiral configuration, and an
77 enantiomeric form may exist with the opposite configuration. For instance, the most famous
78 Whelk-O1 stationary phase designed by Pirkle and Welch [18] can be found in (R,R)
79 configuration (as in Figure 1) or in (S,S) configuration, thereby offering the possibility to
80 control the elution order of enantiomers by choosing one chiral selector or its enantiomeric
81 form. Indeed, when the aim of the chiral separation is to measure an enantiomeric excess,
82 the minor enantiomer should preferably elute first to avoid being masked by the tailing peak
83 of the major enantiomer. On the contrary, when purification is desired, the preferred
84 enantiomer (whichever its proportion) should elute first because the first peak purified is
85 usually obtained with a higher purity than the second. Indeed, although their loadability is not

86 as high as that of polysaccharide CSPs, it is still higher than the loadability of CSPs based on
87 proteins, cyclodextrins and macrocyclic glycopeptides [19], making Pirkle-type CSPs useful
88 for preparative purposes. Stereoisomer identification and measuring extreme enantiomeric
89 excess values (>99%) can be enabled by the possibility to reverse the stereoisomers elution
90 order with chiral selectors of opposite configuration, as was demonstrated with the “Inverted
91 Chirality Columns Approach” proposed by Gasparrini and co-workers [20–22].
92 Following the current trend of reducing particle size, the most recent version of Whelk-O1 for
93 analytical-scale separations is available on sub-2 μm fully porous particles and has
94 demonstrated improved efficiencies, as could be expected [23,24].
95 While Pirkle-type stationary phases were mostly employed in high-performance liquid
96 chromatography (HPLC), they are also useful in supercritical fluid chromatography (SFC),
97 where they are usually found to offer complementary capabilities for enantioselectivity to the
98 most employed polysaccharide CSPs [25]. In this field, the Whelk-O1 phase appears as the
99 most widely distributed brush-type phase. Modern SFC is essentially based on the use of
100 pressurized carbon dioxide as the principal component of the mobile phase, together with a
101 co-solvent (most often a short-chain alcohol) [26,27]. Thanks to several interesting attributes
102 of carbon dioxide (low toxicity, no safety issues, low price) and the inherent advantages of
103 the technique (low-viscosity fluid and high diffusivities providing high efficiencies even at high
104 flow rates, low amount of waste especially after preparative separations), SFC has long been
105 a favourite to achieve enantioseparations [28,29], usually offering higher resolution per unit
106 time than enantioselective HPLC, thanks to high efficiency and high flow rate [30,31].
107 In previous works, we have proposed a chemometrics strategy to improve understanding of
108 SFC enantioseparation mechanisms based on the use of linear solvation energy
109 relationships (LSER) and discriminant analyses (DA) [32,33]. First, we developed a modified
110 version of the solvation parameter model, comprising five Abraham descriptors (E, S, A, B,
111 V) and four additional descriptors that were introduced to bring complementary information
112 related to (i) analyte shape features of flexibility (F) and sphericity (or globularity, G) and (ii)
113 ionic interaction capability through anionic (D^-) and cationic (D^+) analyte properties. With this
114 model, we have previously examined polysaccharide CSPs [34,35], macrocyclic
115 glycopeptide CSPs [36,37] and *Cinchona*-based brush-type CSPs [38], all in SFC
116 conditions. In the present paper, this modified solvation parameter model is employed to
117 characterize the (R,R)-Whelk-O1 enantioselective stationary phase in supercritical fluid
118 chromatography based on the analysis of 212 achiral analytes. A similar phase from a
119 different manufacturer (ReproSil Chiral-NR), carrying the (S,S) enantiomeric ligand from
120 (R,R)-Whelk-O1 was included for comparison purposes, as we had observed in the past that

121 supposedly identical enantioselective stationary phases provided by different manufacturers
122 could sometimes be significantly different [39].

123 Secondly, we have also demonstrated earlier that discriminant analysis (DA) was a useful
124 tool to unravel the contributions of analyte features to enantiorecognition [34–36]. Based on
125 the analysis of 79 chiral analytes, the structural features contributing to favourable
126 enantiorecognition of (R,R)Whelk-O1 in SFC will be discussed.

127

128

129 **3. Material and methods**

130

131 ***3.1. Stationary phases***

132

133 The stationary phases used in this study are commercially available: (R,R)-Whelk-O1 from
134 Regis Technologies was packed in 150 x 4.6 mm column, with fully porous 5 µm silica
135 particles, and ReproSil Chiral-NR (bonded with the (S,S) ligand) from Dr. Maisch was
136 packed 250 x 4.6 mm column, with fully porous 8 µm silica particles.

137

138 ***3.2. Chemicals***

139

140 The solvent used was HPLC-grade methanol (MeOH) provided by VWR (Fontenay-sous-
141 Bois, France). Carbon dioxide of industrial grade 99.5% was provided by Air Liquide
142 (France). 212 achiral test compounds (Table S1 in supplementary information) were obtained
143 from a range of suppliers and were used to evaluate the contributions of molecular features
144 to retention through LSER. 79 chiral racemic test compounds (Table S2 in supplementary
145 information) were used to evaluate the contributions of molecular features to
146 enantiorecognition through DA.

147

148

149 ***3.3. Chromatographic system and conditions***

150

151 Chromatographic separations were carried out using equipment manufactured by Jasco
152 (Tokyo, Japan). Two model 980-PU pumps were used, one for carbon dioxide and a second
153 for the modifier. Control of the mobile phase composition was performed by the modifier
154 pump. The pump head used for pumping the carbon dioxide was cooled to –5 °C by a
155 cryostat (Julabo F10c, Seelbach, Germany). When the two solvents (methanol and CO₂)
156 were mixed, the fluid was introduced into a dynamic mixing chamber PU 4046 (Pye Unicam,

157 Cambridge, UK) connected to a pulsation damper (Sedere, Orleans, France). The columns
158 were thermostated by an oven (Jetstream 2 Plus, Hewlett-Packard, Palo Alto, USA),
159 regulated by a cryostat (Haake D8 GH, Karlsruhe, Germany). The detector was a Gilson UV
160 151 detector (provided by Waters, Milford, MA) equipped with a pressure-resistant cell. After
161 the detector, the outlet column pressure was controlled by a Jasco BP-2080 Plus pressure
162 regulator. The outlet regulator tube (internal diameter 0.25 mm) was heated to 60 °C to avoid
163 ice formation during the CO₂ depressurization. The UV detection wavelength was 210 nm.
164 Chromatograms were recorded using the Azur software (Datalys, France).
165 The mobile phase used in this study is always CO₂-MeOH 90:10 (v/v) without any additive.
166 Flow rate was 3 mL min⁻¹. The oven temperature was set at 25°C and the outlet pressure
167 was maintained at 15 MPa. Strictly speaking, the fluid is thus not supercritical but could be
168 called subcritical. No distinction will be made in the following.

169

170 **3.4. Data analysis**

171

172 The Abraham solute descriptors (E, S, A, B, V) used for LSERs with Eq. (1) were extracted
173 from an in-house database established from all available literature on the solvation
174 parameter model (Tables S1 and S2). The two additional descriptors for charges (D⁻ and D⁺)
175 were calculated based on aqueous pK_a values determined with Chemicalize program and
176 apparent pH 5. See previous works for detailed description [40]. The additional descriptors
177 for flexibility (F) and globularity (G) were computed as previously described [32,33].
178 Multiple linear regressions (MLR) and discriminant analysis (DA) were performed using
179 XLStat 19.03.44850 software (Addinsoft, New York, NY). The quality of the MLR fits was
180 estimated using the adjusted determination coefficient (R²), standard error in the estimate
181 (SE) and Fisher F statistic. The statistical significance of individual coefficients was evaluated
182 with the 95% confidence intervals.

183 The quality of DA was estimated based on ROC (receiver operating characteristics) curves
184 and confusion matrices (see Figure S1 in supplementary information).

185

186 **4. Results and discussion**

187

188 The stationary phases examined in this paper bear the same chiral selector (in two
189 enantiomeric forms), presented in Figure 1. The ligand can be fragmented into three
190 sections: two aromatic rings arranged in a perpendicular orientation, with one being π-donor
191 (the tetrahydrophenanthren ring) and the other one being both a π-acceptor, a dipole-dipole

192 interaction site and a hydrogen bond acceptor site (the 3,5-dinitrobenzoyl ring); between
193 them is an amide function acting as a hydrogen-bond system, which can contribute to
194 hydrogen bonding both as a proton donor (-NH- group) and as a proton acceptor (C=O
195 group). This stationary phase is one of those of which the recognition mechanism has been
196 described with most details [4] and is supposedly one of the easiest to explain (unlike the
197 most complex CSPs based on polysaccharide or macrocyclic glycopeptides). Many of the
198 molecular modelling studies to date were done with the chiral selector placed in a supposed
199 “gas state” (not considering possible mobile phase components) [41], or sometimes in a
200 “liquid state” (considering solvent molecules) [9], and always compared the computational
201 results to liquid chromatography experimental data. In papers from Cann and co-workers
202 relating docking studies [9,10], four interaction modes (M1-M4) were observed to occur
203 between the analytes and the chiral selector. The latter was mostly in one, energetically
204 more stable conformation, but could adopt a slightly different conformation for one interaction
205 mode (M1 “side-of-cleft binding”), with the dinitrophenyl group rotating in a perpendicular
206 position. It was also shown that predicting the outcome of a separation on this stationary
207 phase was possible, with rather high success rate of the prediction [42], which is another
208 indication of a well-defined enantioselective process. Comparison to the knowledge
209 previously acquired with molecular modelling and liquid chromatography, to our results,
210 based on a statistical model and observations done in pressurized carbon dioxide –
211 methanol mobile phase, was also of interest in this study.

212 The sets of achiral and chiral probe analytes were previously designed for the
213 characterization of polysaccharide CSPs. It is worth mentioning that it is still adequate to
214 characterize these brush-type CSPs as appears from Figure 2.

215 First, the retention data of the achiral analytes (Table S1) are adequately scattered in a wide
216 retention space with $\log k$ values ranging from -1 to +2 (Figure 2a). Besides, the retention
217 data between the two columns were highly correlated ($R^2 = 0.97$), but the slope of the
218 correlation line was significantly inferior to 1 (0.80) indicating that the two columns have
219 *homeoenergetic* behaviour, according to the definition proposed by Horváth and co-workers
220 [43], and suggesting similar physico-chemical basis for retention on the two columns. The κ - κ
221 plot ($\log k$ vs. $\log k$) can be observed in Figure S2 provided in supplementary information.
222 The observed slope inferior to 1 may simply result from the different silica particles employed
223 to support the chiral selector. Full information on the silica (pore size, specific surface area)
224 and bonding density was not available, but the two CSPs had different particle size (5 and 8
225 μm).

226 Secondly, the separation data measured for the analytes in Table S2 are also adequately
227 scattered with $\log \alpha$ values ranging from 0 to 0.4 (Figure 2b) and about 60% showing α
228 values >1 . For the purpose of identifying the structural features contributing to

229 enantiorecognition, it is important that the probe set comprises both resolved and non-
230 resolved enantiomers.

231

232 **4.1. Interactions contributing to retention**

233

234 The LSER equation employed in this work was previously described [37]:

$$235 \quad \log k = c + eE + sS + aA + bB + vV + d^-D^- + d^+D^+ + fF + gG \quad (1)$$

236

237 Briefly, the capital letters are molecular descriptors: E is related to the presence of π and n
238 electrons, S is related to the presence of dipoles, A is related to proton-donor capability, B is
239 related to electron-donor capability, V is the molecular volume, D^- is the total negative
240 charge, D^+ is the total positive charge, F qualifies the molecule flexibility and G is the
241 molecule sphericity. The small case letters are the system constants obtained from multiple
242 linear regression of retention data ($\log k$ values) for a large set of analytes with known
243 descriptor values. They quantify the relative contribution of interactions to the retention
244 mechanism.

245

246 The results of LSER characterization are presented in Figure 3 (normalized coefficients), with
247 complete data and statistics available in Table 1. The statistics were reasonably good, with
248 R^2_{adj} values of about 0.85 and standard deviation in the estimate of 0.28 and 0.14 for Whelk-
249 O1 and ReproSil Chiral-NR respectively. These results are clearly not as good as is usually
250 observed when the solvation parameter model is applied for achiral separations, whatever
251 the chromatographic mode, but considering the complexity of the system used here, they
252 should be good enough to interpret the contributions of interactions to retention. The two
253 columns yielded identical models as no significant difference can be observed (considering
254 standard error bars). This is in accordance with the previous observation of high correlation
255 of the retention data (Figure S2).

256 A first observation is that the e term, reflecting interactions associated to the presence of π
257 and n electrons in the analytes, is by far the most significant one to contribute to retention.
258 This was naturally expected, judging from the two aromatic groups (dinitrobenzyl and
259 tetrahydrophenanthryl) in the chiral selector. Halogen bond [44], occurring between halogen
260 atoms of some probe analytes and the nitrogen and oxygen atoms or π -donor system of the
261 chiral selector may also be included in this e term. Typically, retention increase is observed
262 when moving from chloride to bromide and iodide. For instance, this can be observed with
263 halogenobenzenes (analytes #87 to #89 in Table S1) and halogenonaphthalenes (analytes
264 #96 to #98).

265 Next to the e term, hydrogen bonding interactions with proton donors (a term) and acceptors
266 (b term) are the most significant, and are related to the presence of the amide function in the
267 chiral selector, which can interact both with proton donors (a term) and proton acceptors (b
268 term), but also to the presence of nitro groups that can interact with proton donors [8–
269 10,45]. The influence of hydrogen bonding can be observed for instance in the increased
270 retention observed when increasing the number of hydroxyl groups in the analyte, as for
271 phenol, resorcinol and phloroglucinol (analytes #103, 108 and 111 in Table S1), or for any
272 pair of analytes differing from one hydroxyl group (many possible pairs in Table S1).
273 Of slightly inferior significance are the dipole-dipole interactions (s term) related to the amide
274 and nitro functions, and the interactions with cationic species (d^+ term), possibly due to non-
275 bonded silanol groups at the surface of the silica supporting phase. The latter would also
276 cause some repulsion to anionic species, as appears from the negative d^- term. Another
277 possible contribution to the retention of ionic analytes is through the aromatic groups of the
278 chiral selector. Firstly, the nitro functions carry partial negative charges on the oxygen atoms,
279 which could also be responsible for the attraction of cationic analytes and repulsion of
280 anionic analytes. Secondly, the tetrahydrophenanthrene ring is a π -donor system, which
281 could also interact with electron-deficient cationic analytes and be responsible for the positive
282 d^+ term. It is also worth mentioning that the d^-/d^+ terms are not significantly different between
283 the two stationary phases, suggesting that silanol accessibility and/or ligand bonding density
284 should be comparable between the two phases.

285 From these models, analyte size (described by the V descriptor) appears to have no
286 influence on analyte retention. However, the analyte shape described by the G descriptor is
287 significant: spherical molecules (large G values) must be somewhat sterically excluded from
288 the stationary phase, as opposed to flat and/or linear molecules (small G values). Significant
289 examples can be observed with polynuclear aromatic hydrocarbons. For instance, p -
290 terphenyl (analyte #180 in Table S1), a rod-like molecule, is more retained than o -terphenyl
291 (analyte #179) which is more spherical, as indicated by a larger G value. These observations
292 may be somewhat contradicted because there is some inter-correlation between the V and G
293 terms ($R^2 = -0.77$). When retaining only the five Abraham descriptors in the model
294 calculation, the v term is significant and positive, although small, indicating that the retention
295 of large molecules is favoured by (i) interaction with the aromatic rings (dispersive or CH- π
296 interactions), or (ii) to a lesser extent, dispersive interactions with the alkyl spacer arm linking
297 the chiral selector to the silica surface. For instance, increasing alkyl chain length, which is
298 inducing increasing V values while all other molecular descriptors remain virtually constant, is
299 causing a slight increase in retention. Typically, this can be observed in the alkylbenzene
300 series (analytes #2 to #15 in Table S1), phenylalcohol series (analytes #70 to #72),

301 alkylphenone series (analytes #80 to #82), alkylbenzoate series (analytes #152 to #155),
302 dialkylphthalate series (analytes #156 to #159), and parabens (analytes #160 to #163).
303 Finally, flexibility (described by F descriptor) has no influence on retention neither. It was
304 never observed to have an influence on retention on the other CSPs previously tested [34–
305 38], but is retained in the model for its possible contribution to enantiorecognition.
306

307

308 **4.2. Enantioseparation capabilities**

309

310 Because the two stationary phases were observed to be most similar in their retention and
311 separation behaviour, only the results for the Whelk-O1 phase will be detailed in the
312 following.

313

314 In previous works, we have had several occasions to demonstrate a strategy to explain
315 enantioseparation mechanisms with discriminant analyses (DA). Discriminant analysis is a
316 supervised method: the samples (in the present case the racemates) are first classified into
317 two sets of classes (i) based on experimental retention compared to predicted retention
318 (eluted earlier vs. later than predicted), or (ii) based on enantioselectivity ($\alpha = 1$ vs. $\alpha > 1$).
319 The first set of DA classes is based on experimental retention factors measured for the two
320 enantiomers and comparing these experimental values to the retention factor predicted by
321 Eq. (1), with the models calculated above based on the retention of achiral analytes (Table
322 1). Because the descriptors are identical for the two enantiomers, there is only one predicted
323 retention factor for each racemate. Usually, both enantiomers appear to be either less
324 retained than predicted, or more retained than predicted, with one of them being close to the
325 predicted value. In rare cases, the first eluted enantiomer is less retained than predicted
326 while the second eluted enantiomer is more retained than predicted. We then have divided
327 the whole set of racemates (see DA classes in Table S2) into two classes: the “early-eluting
328 racemates” and the “late-eluting racemates”. Discriminant analysis applied to the whole set
329 of racemates, using their molecular descriptors as variables and the two classes thus defined
330 is yielding Figure 4a for Whelk-O1. The AUC (area under the ROC curve, which can be
331 observed in supplementary information Figure S1) was 0.829, indicating that the
332 classification obtained is statistically significant. Because there are only two classes, a single
333 axis is sufficient to represent 100% of the variance. On Figure 4a, the structural features that
334 point to the left of the figure have larger descriptor values in the racemates eluting earlier
335 than predicted, while the structural features that point to the right of the figure have larger
336 descriptor values in the racemates eluting later than predicted.

337 The size and shape features are all significant and can be explained as follows: the late-
338 eluting racemates are mostly small (molecular volume V is pointing to the left) and spherical
339 (globularity G is pointing to the right). The chiral ligand in these stationary phases is known to
340 form a semi-rigid cleft [8]. A large and flat or rod-like molecule (with small sphericity) is
341 unlikely to fit in the chiral cleft but may still be retained (and even resolved) through
342 interactions outside the cleft [46]. For instance, docking studies from Zhao and co-workers
343 [9,10] and from Roussel group [8] showed how the dinitrobenzyl ring could rotate to interact
344 with an analyte that would mostly remain outside the cleft. On the contrary, a small spherical
345 molecule is likely to better fit the walls of the chiral cleft and interact favourably with the
346 different groups of the ligand, favouring stronger retention. This hypothesis is also supported
347 by the observation that nearly all late-eluting racemates (25% of the complete set) showed
348 some enantioselectivity ($\alpha > 1$). In other words, when the enantiomers were favourably
349 included in the chiral cleft, some resolution occurred nearly systematically (19 cases out of
350 20) for this probe set. Metoprolol was the sole exception in this group. The “early-eluting”
351 racemates, supposedly not included in the chiral cleft, showed much lower success rates
352 with about 50% enantiodiscrimination observed.

353 We may also note that most acidic species, bearing some anionic charge in the present
354 operating conditions, also belong to this class of late-eluting racemates (D^- is pointing to the
355 right of the figure). This may be surprising since there are no ion-exchange interaction site
356 bearing a permanent charge in this stationary phase. There are however possible residual
357 silanol groups at the silica surface and the two aromatic groups of the chiral selector, which
358 are electron-rich (tetrahydrophenanthrene) and electron-deficient (dinitrobenzene). Residual
359 silanol groups should exert ionic repulsion to anionic analytes, as suggested from the LSER
360 models (negative d' term). Repulsive interactions with the supporting phase may however be
361 favourable to interaction with the bonded chiral selector, as the analytes would be
362 preferentially retained on the chiral selector rather than penetrating deeper in the stationary
363 phase, thereby promoting enantioselectivity. It was also shown [47] in previous molecular
364 modelling studies on Whelk-O1 that non-enantiospecific interactions could influence the way
365 the analytes were interacting with the chiral selector, thereby influencing the
366 enantioselectivity process. In addition, a negatively charged analyte and the π -acceptor
367 dinitrobenzyl ring of the chiral ligand may interact through anion- π interaction [48]. Such
368 interaction may be favoured by the relative flexibility of the dinitrobenzyl ring, which was
369 observed to adapt its torsion angle to favour analyte binding [8,10]. Further molecular
370 modelling studies would be necessary to support any of these hypotheses.

371
372 The second set of DA classes examined is based on the enantioselectivity. Any racemate
373 where some enantioselectivity is observed ($\alpha > 1$) is classified as “separated” while $\alpha = 1$

374 would be classified as “non-separated”. Processing the whole data set in one discriminant
375 analysis is possible and is yielding reasonably good statistics in this case (AUC = 0.782, see
376 supplementary information Figure S1). The result can be observed in Figure 4b. However, as
377 we have had occasions to demonstrate in the past [34,35], it is beneficial to use the above
378 early/late-eluting classifiers prior to the DA calculation, because different groups of analytes
379 may be resolved for different reasons. As mentioned above, all (but one) racemates eluting
380 later than predicted were “separated”, thus no DA can be computed specifically for this group
381 as a sufficient number of negative cases are required to obtain statistically meaningful
382 results. The group of early-eluting racemates however was still large, comprising 59
383 racemates among which 30 were “not separated” and 29 were “separated”. This is a
384 reasonably large and balanced data set to compute a DA, as the number of analytes is nearly
385 equal in each class. The result was statistically significant (AUC = 0.775, see Figure S1 in
386 supplementary information) and presented in Figure 4c. From this figure, we can note that,
387 when the racemates were early-eluting, even if they were mostly large (V pointing to the
388 right) and flat (or rod-like, G pointing to the left) as was defined in the early/late-eluting DA,
389 they could still be separated if they had π electrons to interact with the aromatic rings of the
390 chiral selector (E pointing to the right), dipoles to interact with the nitro groups or amide
391 group of the chiral selector (S pointing to the right) and electron-donating capability to form
392 hydrogen bonds with the –NH– group in the amide function (B pointing to the right). The
393 significant contribution of E, S, and B for this group is consistent with previous observations
394 [9,10], where molecular modelling indicated that many of the less retained analytes were
395 interacting with the chiral selector through the aromatic groups and through hydrogen
396 bonding with the amide proton. Other features (proton-donor capability A, presence of
397 negative or positive charges D^- and D^+ and flexibility F) were non-significant to explain the
398 differences between the two classes. This does not mean that they do not contribute to any
399 interactions but only that they were not sufficiently different between the two classes. For
400 instance, proton-donors (a term) and cationic species (d^+ term) were retained (according to
401 LSER model in Figure 3) but they were present equally in both racemate classes
402 (“separated” and “non-separated”). As a result the A term and D^+ term are both close to zero
403 in the discriminant analysis (Figure 4c). The non-discriminant contribution of A term is
404 consistent with the observed orientation of the carbonyl group towards the exterior of the
405 chiral cleft [5], and is also consistent with previous molecular modelling studies, where
406 hydrogen bonding between an analyte proton and the carbonyl or nitro functions of the chiral
407 selector were observed to be much less frequent than the hydrogen bonding to the amide
408 proton [8–10]. The interactions with cationic species could result from the presence of non-
409 bonded silanol groups, which should also be non-enantioselective, or from interactions with
410 the two aromatic groups (tetrahydrophenantrenyl and dinitrobenzyl), as mentioned in the

411 description of retention models. Most anionic species were included in the late-eluting class
412 thus this feature (D^-) could not be evaluated in the early-eluting class where most analytes
413 had zero D^- value.

414
415 Finally, it is interesting to note that flexibility (F term) was non-discriminant. This is rather
416 surprising because flexibility was found to be very significant as an unfavourable feature to
417 enantio recognition with other CSPs previously characterized, particularly with polysaccharide
418 CSPs and *Cinchona*-based brush-type CSPs [38]. Flexibility is usually deleterious to
419 enantio recognition because flexible molecules have more conformers, that make the two
420 enantiomers less easily discriminated. Also, as pointed out by Lämmerhofer [49], rigid
421 molecules require less interactions to achieve enantioselectivity as they meet the geometrical
422 requirements easier than flexible molecules. From the discriminant analyses presented here,
423 this is clearly not an issue on these Pirkle-type CSPs. The supposedly negative effects of
424 flexibility may be contradicted by a positive dynamic fit, whereby the interactions between the
425 flexible analytes and the ligand are favoured by conformational adaptation. It is possible that
426 this flexible adaptation of the analyte would be facilitated by the semi-rigid structure of the
427 chiral selector in this Pirkle-type phase, compared to more flexible CSPs (like those based on
428 polysaccharides).

429
430 Considering all information from the retention models and discriminant analyses, Figure 5
431 provides another look at the retention and separation data, in conjunction with structural
432 features. Figure 5 is plotting $\log \alpha$ between the two enantiomers versus $\log k$ of the first
433 eluted enantiomer. Each point thus represents one racemate, while the bubble size is
434 reflecting one structural feature. For instance, π -electrons (E), dipoles (S), sphericity (G) and
435 cationic charges (D^+) are represented. On the top figures, it appears that the smallest values
436 of E and S (small bubbles) are associated to small retention factors (in accordance with the
437 retention models in Figure 3) and small separation factors (in accordance with Figure 4c). In
438 the top right corner of these figures, the bigger bubbles indicate that the molecules that are
439 π -electron-rich (large E values) and/or possess strong dipoles (large S values) have both
440 strong retention and large enantioresolution. According to the observations in Figure 4, these
441 may belong to the late-eluting class, if they are rather small (small V value), spherical (large
442 G value) and possess negative charges (non-zero D^- value) through acidic functions. Typical
443 examples are 2-phenylbutyric acid (analyte #2 in Table S2) and 2-phenylpropionic acid (#3).
444 In addition, enantiomers with large E and/or S features and successful enantio separation
445 may also belong to the early-eluting class: Figure 4c indicates that favourable
446 enantio recognition in this case will also depend on the ability to form hydrogen bonds (B
447 term) and the size and shape features, as big (large V value) and flat or rod-like (small G

448 value) molecules should be favoured. Typical examples are found in the group of β -blockers
449 Acebutolol (analyte #25 in Table S2), Atenolol (#27), Betaxolol (#28), Oxprenolol (#58),
450 Propranolol (#60) and Sotalol (#63). All of them have several heteroatoms with non-binding
451 electrons contributing to large S and B values, and they have long chains inducing both large
452 volume and rod-like shape. Another example is that of benzodiazepines Diazepam (#35),
453 Lorazepam (#49) and Oxazepam (#56) that are all rather large and flat.

454 However, strong E and S features are not sufficient to achieve enantioseparation, as can be
455 observed with some large bubbles at the bottom line of these figures, corresponding to zero
456 $\log \alpha$ values. As mentioned above, no enantioresolution was principally observed in the
457 early-eluting racemates. Thus analytes with large E and S values eluting earlier than
458 expected may still be unresolved, particularly if they are rather small (small V value) and
459 spherical (large G value). Typical examples are found in the group of barbiturates like
460 Glutethimide (#42), Hexobarbital (#43), Secobarbital (#62) and Thiopental (#67): although
461 they have favourable electron and dipole features (E, S and B values), they are all rather
462 small and spherical and remain unresolved.

463 The bottom left figure in Figure 5 shows that spherical molecules (large G values) are mostly
464 distributed in low retention values (in accordance with Figure 3, indicating exclusion of
465 spherical molecules). However, small spherical molecules (case of "late-eluting racemates")
466 that are little retained are well separated as appears with positive values of $\log \alpha$ on the left
467 of the figure. Again, this would be the case of 2-phenylbutyric and 2-phenylpropionic acids.
468 Non-spherical molecules (flat or rod-like) are more retained and can still be well separated
469 with favourable E, S and B features and a large molecular volume (case of "early-eluting
470 racemates" in Figure 4c). For instance, this is corresponding to the β -blockers and
471 benzodiazepines.

472 Finally, the case of cationic species is also interesting (bottom right figure). Cations (large D^+
473 values) are positively retained on this stationary phase (in accordance with Figure 3,
474 indicating favourable retention of cations), thus the large bubbles are mostly on the right side
475 of this figure. However, the presence of cationic charges was non-discriminant to explain
476 enantioseparation (Figure 4b and 4c), thus big bubbles can be found at all levels of α values.

477

478

479 **5. Conclusions**

480

481 Two Pirkle-type chiral stationary phases bearing the same (but enantiomeric) chiral selector
482 from two different manufacturers were characterized for their retention properties through
483 linear solvation energy relationships and for their enantioseparation properties through

484 discriminant analyses. The two stationary phases appeared to be most similar, with
485 *homeoenergetic* behaviour. π - π interactions were the most significant to explain retention,
486 followed by hydrogen bonding, dipole-dipole interactions and ionic interactions. Steric
487 exclusion was also significant to explain lack of retention for spherical molecules. The
488 racemates possessing favourable features for enantioseparation can be divided into two
489 groups: small spherical molecules that would well fit in the chiral cleft were nearly always
490 resolved, especially if they possessed negative charges. For large and flat or rod-like
491 molecules that are unlikely to fit so well in the chiral cleft, π - π interactions, dipole-dipole
492 interactions and electron-donating properties were most significant to achieve
493 enantioresolution. These observations are in accordance with chemical sense and previous
494 investigations on these chiral selectors.

495

496

497 **Acknowledgment**

498 Jelena Kocergin (formerly at Regis Technologies) and Albin Maisch (Dr. Maisch) are
499 acknowledged for the kind gift of a column for this study. CW also thanks the Institut
500 Universitaire de France (IUF), of which she is a junior member, for the support received.

501

502 **References**

503

- 504 [1] F. Gasparrini, D. Misiti, C. Villani, High-performance liquid chromatography
505 chiral stationary phases based on low-molecular-mass selectors, *J. Chromatogr.*
506 *A.* 906 (2001) 35–50. [https://doi.org/10.1016/S0021-9673\(00\)00953-5](https://doi.org/10.1016/S0021-9673(00)00953-5).
- 507 [2] J. Haginaka, Protein-based chiral stationary phases for high-performance liquid
508 chromatography enantioseparations, *J. Chromatogr. A.* 906 (2001) 253–273.
509 [https://doi.org/10.1016/S0021-9673\(00\)00504-5](https://doi.org/10.1016/S0021-9673(00)00504-5).
- 510 [3] V.A. Davankov, Chiral selectors with chelating properties in liquid
511 chromatography: Fundamental reflections and selective review of recent
512 developments, *J. Chromatogr. A.* 666 (1994) 55–76.
513 [https://doi.org/10.1016/0021-9673\(94\)80370-6](https://doi.org/10.1016/0021-9673(94)80370-6).
- 514 [4] C.J. Welch, Evolution of chiral stationary phase design in the Pirkle laboratories,
515 *J. Chromatogr. A.* 666 (1994) 3–26. [https://doi.org/10.1016/0021-](https://doi.org/10.1016/0021-9673(94)80367-6)
516 [9673\(94\)80367-6](https://doi.org/10.1016/0021-9673(94)80367-6).
- 517 [5] C. Fernandes, M.E. Tiritan, M. Pinto, Small Molecules as Chromatographic
518 Tools for HPLC Enantiomeric Resolution: Pirkle-Type Chiral Stationary Phases
519 Evolution, *Chromatographia.* 76 (2013) 871–897.
520 <https://doi.org/10.1007/s10337-013-2469-8>.
- 521 [6] L.H. Easson, E. Stedman, Studies on the relationship between chemical
522 constitution and physiological action, *Biochem. J.* 27 (1933) 1257–1266.
523 <https://doi.org/10.1042/bj0271257>.
- 524 [7] C.E. Dalglish, 756. The optical resolution of aromatic amino-acids on paper
525 chromatograms, *J. Chem. Soc. Resumed.* (1952) 3940.
526 <https://doi.org/10.1039/jr9520003940>.
- 527 [8] A. Del Rio, J.M. Hayes, M. Stein, P. Piras, C. Roussel, Theoretical
528 reassessment of Whelk-O1 as an enantioselective receptor for 1-(4-halogeno-
529 phenyl)-1-ethylamine derivatives, *Chirality.* 16 (2004) S1–S11.
530 <https://doi.org/10.1002/chir.20009>.
- 531 [9] C.F. Zhao, N.M. Cann, Molecular Dynamics Study of Chiral Recognition for the
532 Whelk-O1 Chiral Stationary Phase, *Anal. Chem.* 80 (2008) 2426–2438.
533 <https://doi.org/10.1021/ac702126y>.
- 534 [10] C.F. Zhao, S. Diemert, N.M. Cann, Rational optimization of the Whelk-O1 chiral
535 stationary phase using molecular dynamics simulations, *J. Chromatogr. A.* 1216
536 (2009) 5968–5978. <https://doi.org/10.1016/j.chroma.2009.06.041>.
- 537 [11] M.E. Koscho, P.L. Spence, W.H. Pirkle, Chiral recognition in the solid state:
538 crystallographically characterized diastereomeric co-crystals between a
539 synthetic chiral selector (Whelk-O1) and a representative chiral analyte,
540 *Tetrahedron Asymmetry.* 16 (2005) 3147–3153.
541 <https://doi.org/10.1016/j.tetasy.2005.08.027>.
- 542 [12] B. Chankvetadze, Recent developments on polysaccharide-based chiral
543 stationary phases for liquid-phase separation of enantiomers, *J. Chromatogr. A.*
544 1269 (2012) 26–51. <https://doi.org/10.1016/j.chroma.2012.10.033>.
- 545 [13] G.K.E. Scriba, Chiral recognition in separation sciences. Part I: Polysaccharide
546 and cyclodextrin selectors, *TrAC Trends Anal. Chem.* 120 (2019) 115639.
547 <https://doi.org/10.1016/j.trac.2019.115639>.
- 548 [14] J. Shen, Y. Okamoto, 8.11 Chromatographic Separations and Analysis:
549 Cellulose and Polysaccharide Derivatives as Stationary Phases, in: *Compr.*

- 550 Chirality, Elsevier, 2012: pp. 200–226. [https://doi.org/10.1016/B978-0-08-](https://doi.org/10.1016/B978-0-08-095167-6.00824-7)
551 [095167-6.00824-7](https://doi.org/10.1016/B978-0-08-095167-6.00824-7).
- 552 [15] G.K.E. Scriba, Chiral recognition in separation sciences. Part II: Macrocyclic
553 glycopeptide, donor-acceptor, ion-exchange, ligand-exchange and micellar
554 selectors, *TrAC Trends Anal. Chem.* 119 (2019) 115628.
555 <https://doi.org/10.1016/j.trac.2019.115628>.
- 556 [16] I. Ilisz, R. Berkecz, A. Péter, Retention mechanism of high-performance liquid
557 chromatographic enantioseparation on macrocyclic glycopeptide-based chiral
558 stationary phases, *J. Chromatogr. A.* 1216 (2009) 1845–1860.
559 <https://doi.org/10.1016/j.chroma.2008.08.041>.
- 560 [17] A. Berthod, 8.12 Chromatographic Separations and Analysis: Macrocyclic
561 Glycopeptide Chiral Stationary Phases, in: *Compr. Chirality*, Elsevier, 2012: pp.
562 227–262. <https://doi.org/10.1016/B978-0-08-095167-6.00825-9>.
- 563 [18] W.H. Pirkle, C.J. Welch, Use of simultaneous face to face and face to edge π - π
564 interactions to facilitate chiral recognition, *Tetrahedron Asymmetry.* 5 (1994)
565 777–780. [https://doi.org/10.1016/S0957-4166\(00\)86225-4](https://doi.org/10.1016/S0957-4166(00)86225-4).
- 566 [19] E.R. Francotte, Enantioselective chromatography as a powerful alternative for
567 the preparation of drug enantiomers, *J. Chromatogr. A.* 906 (2001) 379–397.
568 [https://doi.org/10.1016/S0021-9673\(00\)00951-1](https://doi.org/10.1016/S0021-9673(00)00951-1).
- 569 [20] E. Badaloni, W. Cabri, A. Ciogli, R. Deias, F. Gasparrini, F. Giorgi, A. Vigevani,
570 C. Villani, Combination of HPLC “Inverted Chirality Columns Approach” and
571 MS/MS Detection for Extreme Enantiomeric Excess Determination Even in
572 Absence of Reference Samples. Application to Camptothecin Derivatives, *Anal.*
573 *Chem.* 79 (2007) 6013–6019. <https://doi.org/10.1021/ac070776j>.
- 574 [21] E. Badaloni, W. Cabri, A. Ciogli, I. D’Acquarica, R. Deias, F. Gasparrini, F.
575 Giorgi, D. Kotoni, C. Villani, Extending the use of “Inverted Chirality Columns
576 Approach” for enantiomeric excess determination in absence of reference
577 samples: Application to a water-soluble camptothecin derivative, *J. Chromatogr.*
578 *A.* 1217 (2010) 1024–1032. <https://doi.org/10.1016/j.chroma.2009.10.035>.
- 579 [22] G. Mazzocanti, O.H. Ismail, I. D’Acquarica, C. Villani, C. Manzo, M. Wilcox, A.
580 Cavazzini, F. Gasparrini, Cannabis through the looking glass: chemo- and
581 enantio-selective separation of phytocannabinoids by enantioselective ultra high
582 performance supercritical fluid chromatography, *Chem. Commun.* 53 (2017)
583 12262–12265. <https://doi.org/10.1039/C7CC06999E>.
- 584 [23] L. Sciascera, O. Ismail, A. Ciogli, D. Kotoni, A. Cavazzini, L. Botta, T. Szczerba,
585 J. Kocergin, C. Villani, F. Gasparrini, Expanding the potential of chiral
586 chromatography for high-throughput screening of large compound libraries by
587 means of sub-2 μ m Whelk-O 1 stationary phase in supercritical fluid conditions,
588 *J. Chromatogr. A.* 1383 (2015) 160–168.
589 <https://doi.org/10.1016/j.chroma.2015.01.042>.
- 590 [24] O.H. Ismail, G.L. Losacco, G. Mazzocanti, A. Ciogli, C. Villani, M. Catani, L.
591 Pasti, S. Anderson, A. Cavazzini, F. Gasparrini, Unmatched Kinetic Performance
592 in Enantioselective Supercritical Fluid Chromatography by Combining Latest
593 Generation Whelk-O1 Chiral Stationary Phases with a Low-Dispersion in-House
594 Modified Equipment, *Anal. Chem.* 90 (2018) 10828–10836.
595 <https://doi.org/10.1021/acs.analchem.8b01907>.
- 596 [25] C.J. Welch, M. Biba, J.R. Gouker, G. Kath, P. Augustine, P. Hosek, Solving
597 multicomponent chiral separation challenges using a new SFC tandem column
598 screening tool, *Chirality.* 19 (2007) 184–189. <https://doi.org/10.1002/chir.20357>.

- 599 [26] E. Lesellier, C. West, The many faces of packed column supercritical fluid
600 chromatography - A critical review, *J. Chromatogr. A.* 1382 (2015) 2–46.
601 <https://doi.org/10.1016/j.chroma.2014.12.083>.
- 602 [27] C. West, Current trends in supercritical fluid chromatography, *Anal. Bioanal.*
603 *Chem.* 410 (2018) 6441–6457. <https://doi.org/10.1007/s00216-018-1267-4>.
- 604 [28] C. West, Enantioselective Separations with Supercritical Fluids - Review, *Curr.*
605 *Anal. Chem.* 10 (2014) 99–120. <https://doi.org/10.2174/1573411011410010009>.
- 606 [29] C. West, Recent trends in chiral supercritical fluid chromatography, *TrAC Trends*
607 *Anal. Chem.* 120 (2019) 115648. <https://doi.org/10.1016/j.trac.2019.115648>.
- 608 [30] S. Khater, M.-A. Lozac'h, I. Adam, E. Francotte, C. West, Comparison of liquid
609 and supercritical fluid chromatography mobile phases for enantioselective
610 separations on polysaccharide stationary phases, *J. Chromatogr. A.* 1467 (2016)
611 463–472. <https://doi.org/10.1016/j.chroma.2016.06.060>.
- 612 [31] R.W. Stringham, Relationship between resolution and analysis time in chiral
613 subcritical fluid chromatography, *Chirality.* 8 (1996) 249–257.
614 [https://doi.org/10.1002/\(SICI\)1520-636X\(1996\)8:3<249::AID-CHIR4>3.0.CO;2-](https://doi.org/10.1002/(SICI)1520-636X(1996)8:3<249::AID-CHIR4>3.0.CO;2-C)
615 *C.*
- 616 [32] C. West, Y. Zhang, L. Morin-Allory, Insights into chiral recognition mechanisms
617 in supercritical fluid chromatography. I. Non-enantiospecific interactions
618 contributing to the retention on tris-(3,5-dimethylphenylcarbamate) amylose and
619 cellulose stationary phases, *J. Chromatogr. A.* 1218 (2011) 2019–2032.
620 <https://doi.org/10.1016/j.chroma.2010.11.084>.
- 621 [33] C. West, G. Guenegou, Y. Zhang, L. Morin-Allory, Insights into chiral recognition
622 mechanisms in supercritical fluid chromatography. II. Factors contributing to
623 enantiomer separation on tris-(3,5-dimethylphenylcarbamate) of amylose and
624 cellulose stationary phases, *J. Chromatogr. A.* 1218 (2011) 2033–2057.
625 <https://doi.org/10.1016/j.chroma.2010.11.085>.
- 626 [34] S. Khater, Y. Zhang, C. West, Insights into chiral recognition mechanism in
627 supercritical fluid chromatography III. Non-halogenated polysaccharide
628 stationary phases, *J. Chromatogr. A.* 1363 (2014) 278–293.
629 <https://doi.org/10.1016/j.chroma.2014.06.084>.
- 630 [35] S. Khater, Y. Zhang, C. West, Insights into chiral recognition mechanism in
631 supercritical fluid chromatography IV. Chlorinated polysaccharide stationary
632 phases, *J. Chromatogr. A.* 1363 (2014) 294–310.
633 <https://doi.org/10.1016/j.chroma.2014.06.026>.
- 634 [36] S. Khater, C. West, Characterization of three macrocyclic glycopeptide
635 stationary phases in supercritical fluid chromatography, *J. Chromatogr. A.* 1604
636 (2019) 460485. <https://doi.org/10.1016/j.chroma.2019.460485>.
- 637 [37] A. Raimbault, C. West, Effects of high concentrations of mobile phase additives
638 on retention and separation mechanisms on a teicoplanin aglycone stationary
639 phase in supercritical fluid chromatography, *J. Chromatogr. A.* 1604 (2019)
640 460494. <https://doi.org/10.1016/j.chroma.2019.460494>.
- 641 [38] A. Raimbault, C.M.A. Ma, M. Ferri, S. Bäurer, P. Bonnet, S. Bourg, M.
642 Lämmerhofer, C. West, Cinchona-based zwitterionic stationary phases:
643 Exploring retention and enantioseparation mechanisms in supercritical fluid
644 chromatography with a fragmentation approach, *J. Chromatogr. A.* 1612 (2020)
645 460689. <https://doi.org/10.1016/j.chroma.2019.460689>.
- 646 [39] S. Khater, Y. Zhang, C. West, In-depth characterization of six cellulose tris-(3,5-
647 dimethylphenylcarbamate) chiral stationary phases in supercritical fluid

- 648 chromatography, *J. Chromatogr. A.* 1303 (2013) 83–93.
649 <https://doi.org/10.1016/j.chroma.2013.06.040>.
- 650 [40] C. West, E. Lemasson, S. Khater, E. Lesellier, An attempt to estimate ionic
651 interactions with phenyl and pentafluorophenyl stationary phases in supercritical
652 fluid chromatography, *J. Chromatogr. A.* 1412 (2015) 126–138.
653 <https://doi.org/10.1016/j.chroma.2015.08.009>.
- 654 [41] K.B. Lipkowitz, Theoretical studies of brush-type chiral stationary phases, *J.*
655 *Chromatogr. A.* 666 (1994) 493–503. [https://doi.org/10.1016/0021-](https://doi.org/10.1016/0021-9673(94)80411-7)
656 [9673\(94\)80411-7](https://doi.org/10.1016/0021-9673(94)80411-7).
- 657 [42] R. Sheridan, W. Schafer, P. Piras, K. Zawatzky, E.C. Sherer, C. Roussel, C.J.
658 Welch, Toward structure-based predictive tools for the selection of chiral
659 stationary phases for the chromatographic separation of enantiomers, *J.*
660 *Chromatogr. A.* 1467 (2016) 206–213.
661 <https://doi.org/10.1016/j.chroma.2016.05.066>.
- 662 [43] W. Melander, J. Stoveken, C. Horváth, Stationary phase effects in reversed-
663 phase chromatography, *J. Chromatogr. A.* 199 (1980) 35–56.
664 [https://doi.org/10.1016/S0021-9673\(01\)91360-3](https://doi.org/10.1016/S0021-9673(01)91360-3).
- 665 [44] R. Dallochio, A. Dessì, M. Solinas, A. Arras, S. Cossu, E. Aubert, V. Mamane,
666 P. Peluso, Halogen bond in high-performance liquid chromatography
667 enantioseparations: Description, features and modelling, *J. Chromatogr. A.* 1563
668 (2018) 71–81. <https://doi.org/10.1016/j.chroma.2018.05.061>.
- 669 [45] W.F. Baitinger, P. von R. Schleyer, T.S.S.R. Murty, L. Robinson, Nitro groups as
670 proton acceptors in hydrogen bonding, *Tetrahedron.* 20 (1964) 1635–1647.
671 [https://doi.org/10.1016/S0040-4020\(01\)99161-6](https://doi.org/10.1016/S0040-4020(01)99161-6).
- 672 [46] M.E. Koscho, W.H. Pirkle, Investigation of a broadly applicable chiral selector
673 used in enantioselective chromatography (Whelk-O 1) as a chiral solvating agent
674 for NMR determination of enantiomeric composition, *Tetrahedron Asymmetry.*
675 16 (2005) 3345–3351. <https://doi.org/10.1016/j.tetasy.2005.08.032>.
- 676 [47] A. Knežević, J. Novak, V. Vinković, New Brush-Type Chiral Stationary Phases
677 for Enantioseparation of Pharmaceutical Drugs, *Molecules.* 24 (2019) 823.
678 <https://doi.org/10.3390/molecules24040823>.
- 679 [48] P. Gamez, T.J. Mooibroek, S.J. Teat, J. Reedijk, Anion Binding Involving π -
680 Acidic Heteroaromatic Rings, *Acc. Chem. Res.* 40 (2007) 435–444.
681 <https://doi.org/10.1021/ar7000099>.
- 682 [49] M. Lämmerhofer, Chiral recognition by enantioselective liquid chromatography:
683 Mechanisms and modern chiral stationary phases, *J. Chromatogr. A.* 1217
684 (2010) 814–856. <https://doi.org/10.1016/j.chroma.2009.10.022>.
- 685

686 **Figure Caption**

687

688 **Figure 1.** Structure of the chiral selector in the (R,R)-Whelk-O1 stationary phase and
689 features of the different fragments of the chiral selector. The ligand in ReproSil
690 Chiral-NR is the (S,S) enantiomer of this one.

691

692 **Figure 2.** Scattergrams and box-plots of (a) retention factors for the 212 achiral
693 analytes in Table S1 and (b) separation factors for the 79 racemates in Table S2 on
694 (R,R)-Whelk-O1. Chromatographic conditions: CO₂-methanol 90:10 (v/v), 25°C, 15
695 MPa, 3 mL/min.

696

697 **Figure 3.** Histogram representing normalized coefficients of the LSER model
698 calculated with the retention data measured for the 212 analytes in Table S1 and Eq.
699 (1) on the (R,R)-Whelk-O1 column (left bars) and ReproSil Chiral-NR column (right
700 bars). Error bars indicate the 95% confidence limits. Chromatographic conditions:
701 see Figure 2.

702

703 **Figure 4.** Discriminant analyses using nine molecular descriptors as variables and
704 the 79 racemates eluted from (R,R)-Whelk-O1 in Table S2.

705 (a) Discrimination between early-eluting (left) and late-eluting (right) racemates
706 indicating the structural features favouring some form of exclusion from the stationary
707 phase (left) or inclusion (right).

708 (b) & (c) Discrimination between non-separated (left) and separated (right) racemates
709 where negative features are common to the racemates that were not separated on
710 this stationary phase and positive features are common to separated racemates. (b)
711 including all racemates (c) including only early-eluting racemates.

712 Chromatographic conditions: see Figure 2.

713

714 **Figure 5.** Bubble graphs to compare retention and separation values for the chiral
715 analytes in Table S2 depending on their structural features. Large bubbles indicate
716 large values for each molecular descriptor.

717 Chromatographic conditions : see Figure 2.

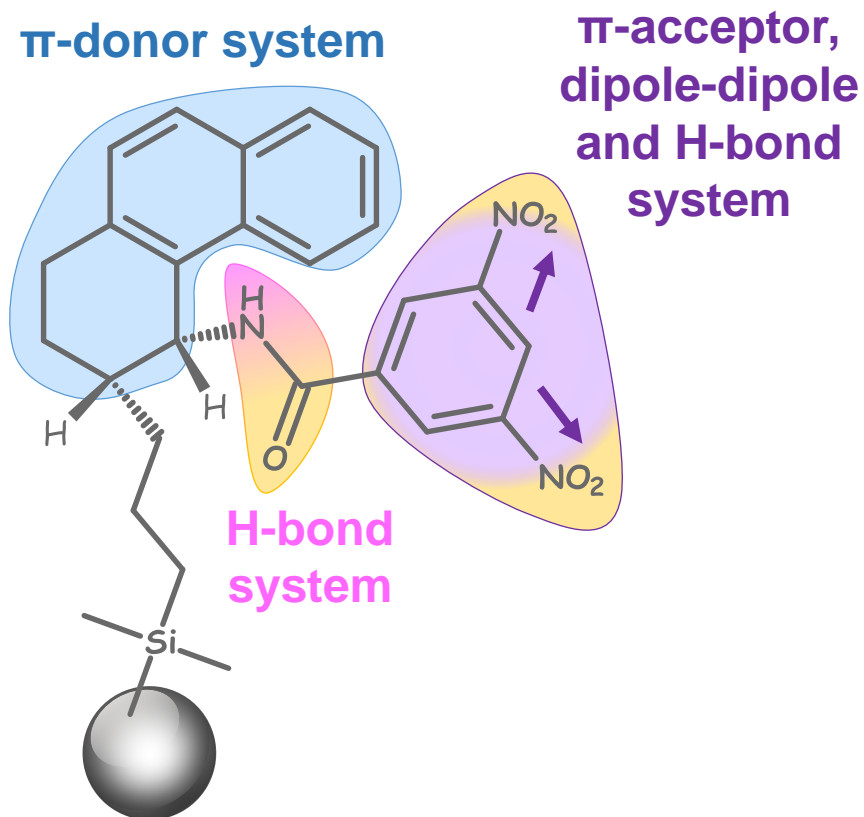


Figure 1: Structure of the chiral selector in the (R,R)-Whelk-O1 stationary phase and features of the different fragments of the chiral selector. The ligand in ReproSil Chiral-NR is the (S,S) enantiomer of this one.

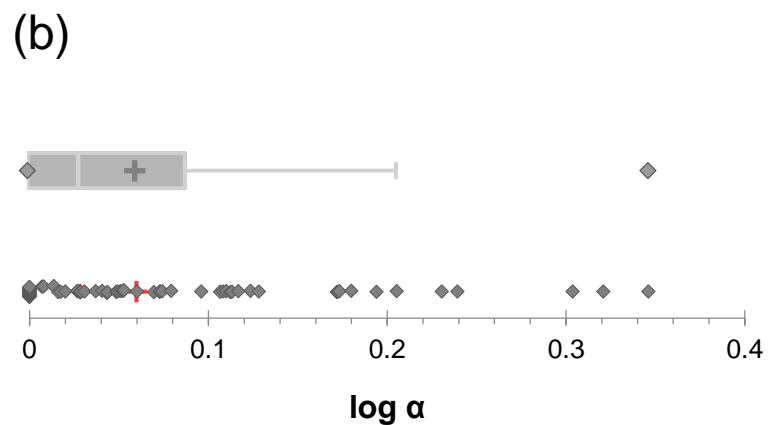
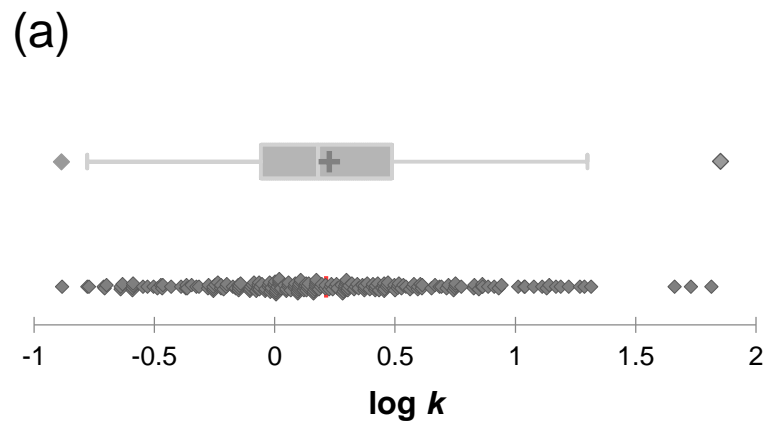


Figure 2: Scattergrams and box-plots of (a) retention factors for the 212 achiral analytes in Table S1 and (b) separation factors for the 79 racemates in Table S2 on (R,R)-Whelk-O1. Chromatographic conditions: CO₂-methanol 90:10 (v/v), 25°C, 15 MPa, 3 mL/min.

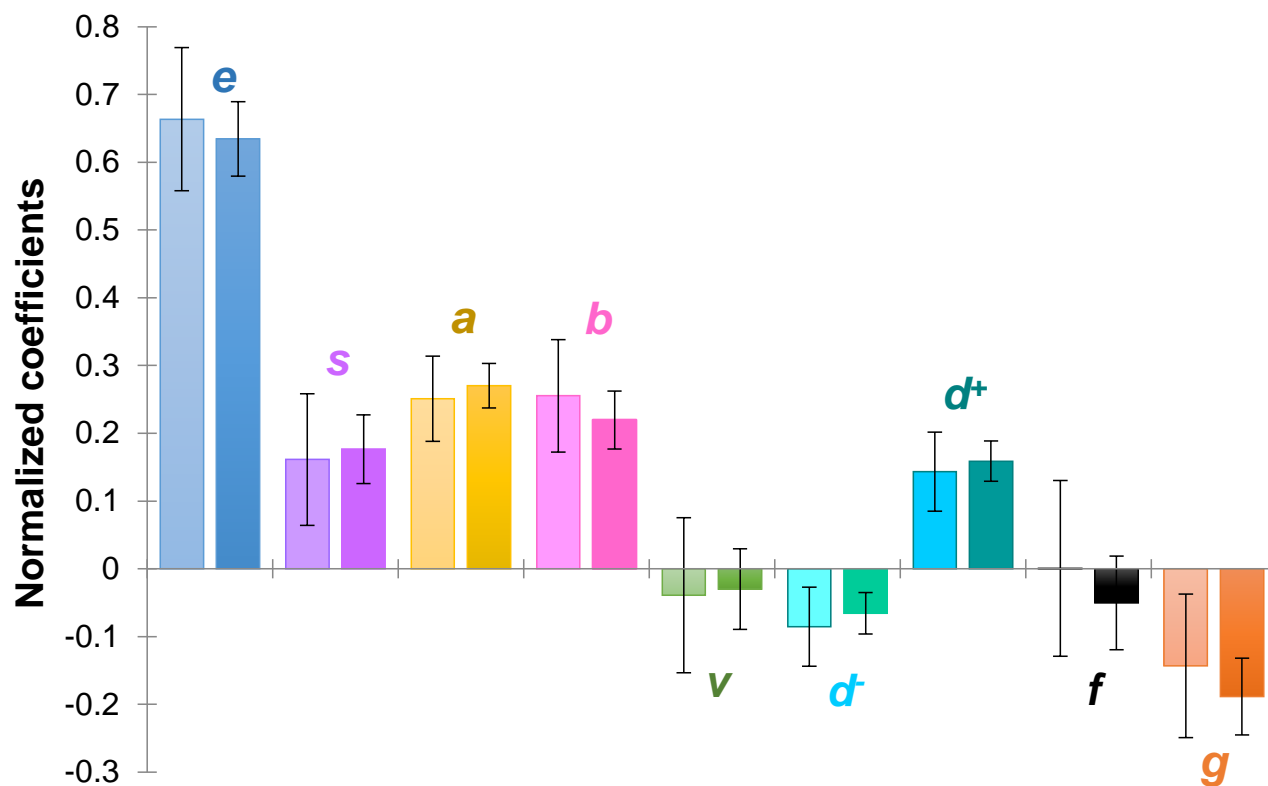


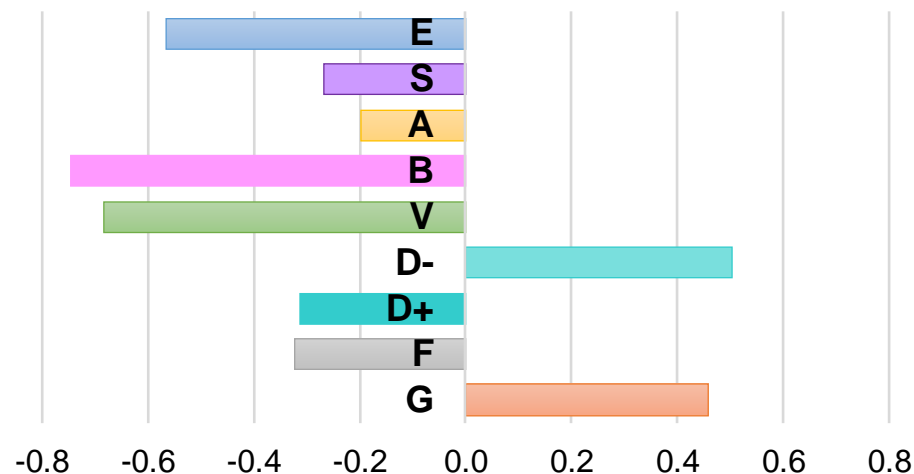
Figure 3: Histogram representing normalized coefficients of the LSER model calculated with the retention data measured for the 212 analytes in Table S1 and Eq. (1) on the Whelk-O1 column (left bars) and Reprisil Chiral-NR column (right bars). Error bars indicate the 95% confidence limits. Chromatographic conditions: see Figure 2.

Figure 4: Discriminant analyses using nine molecular descriptors as variables and the 79 racemates eluted from (R,R)-Whelk-O1 in Table S2.

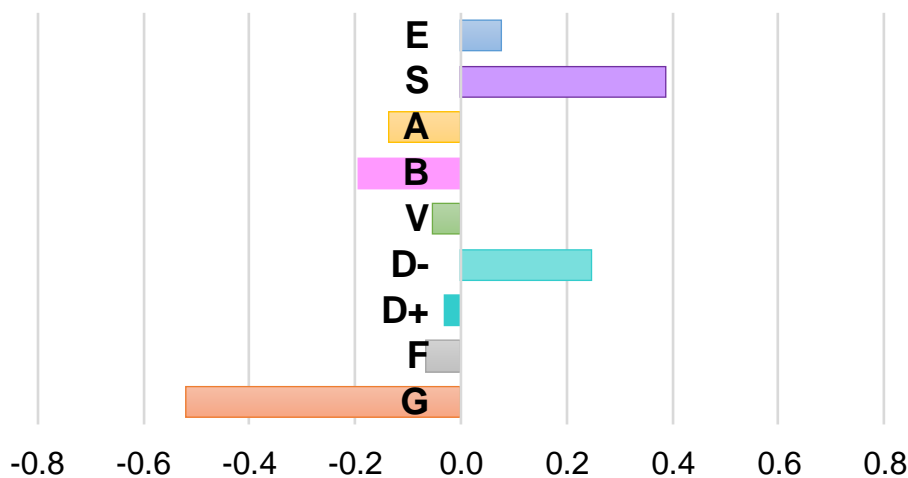
(a) Discrimination between early-eluting (left) and late-eluting (right) racemates indicating the structural features favouring some form of exclusion from the stationary phase (left) or inclusion (right).

(b) & (c) Discrimination between non-separated (left) and separated (right) racemates where negative features are common to the racemates that were not separated on this stationary phase and positive features are common to separated racemates. (b) including all racemates (c) including only early-eluting racemates.

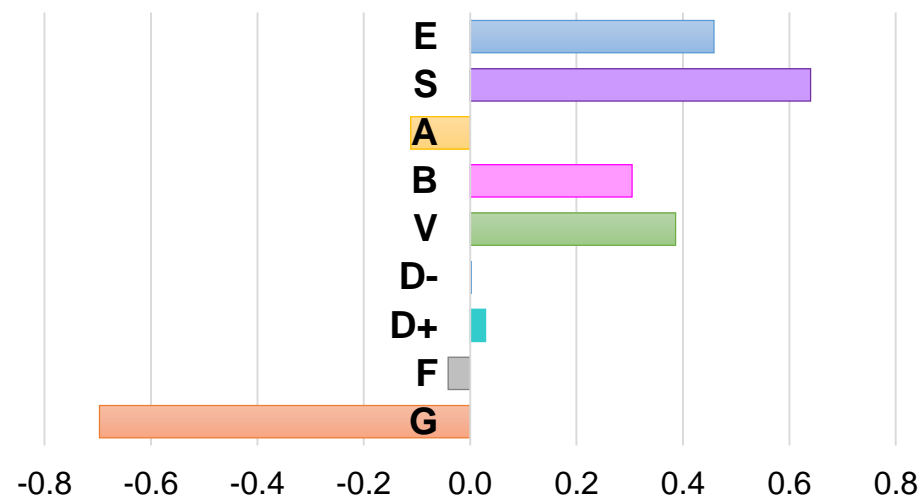
(a) Early-eluting Late-eluting racemates



(b) Non separated Separated racemates



(c) Non separated Separated racemates



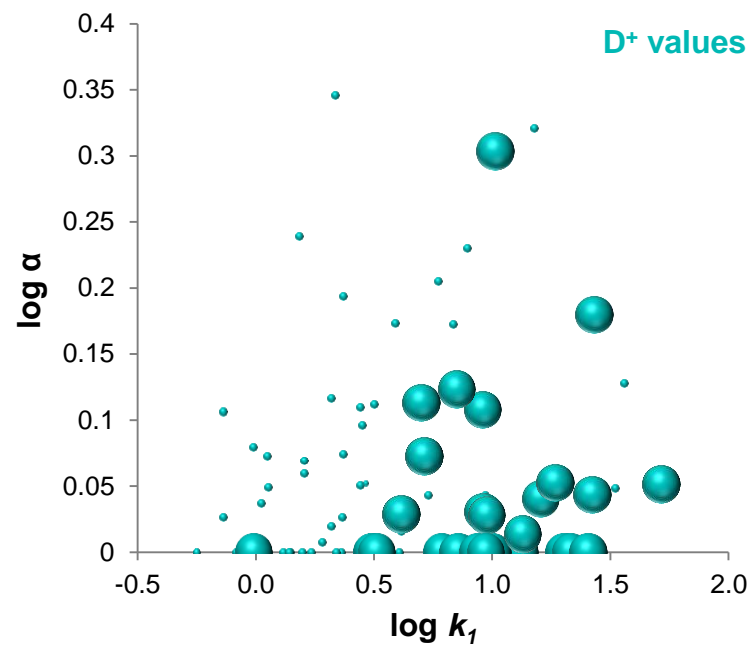
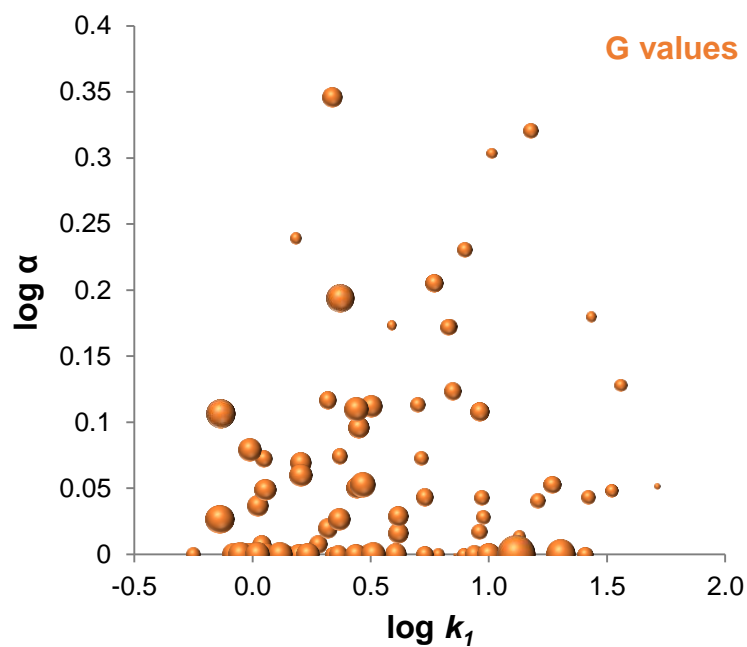
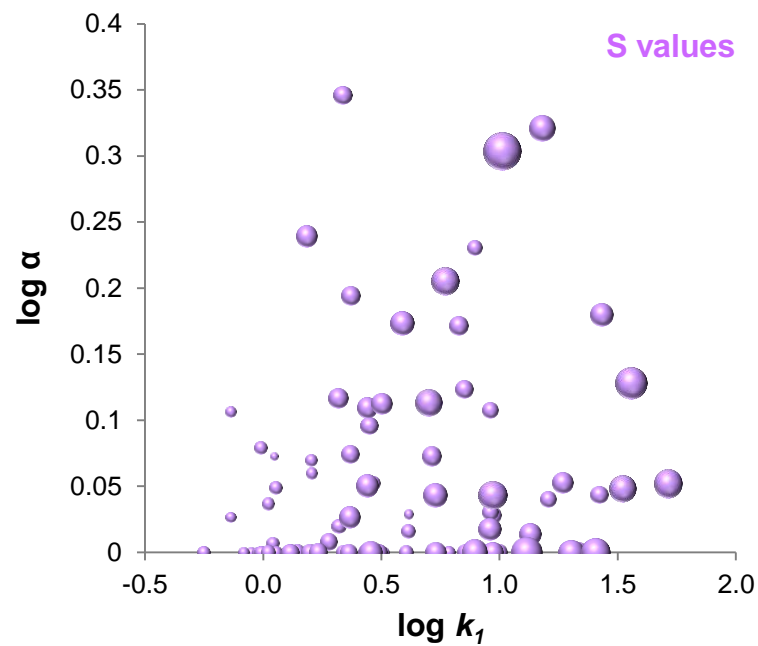
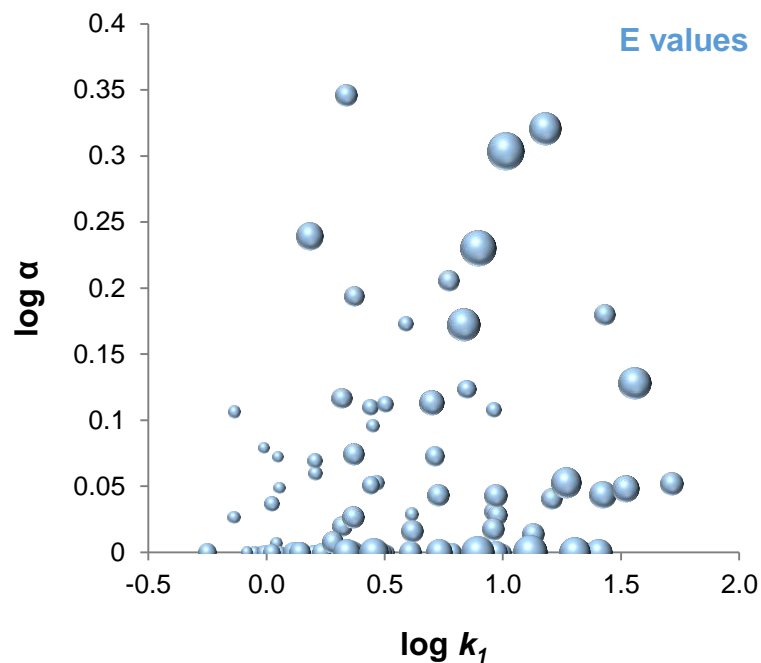


Figure 5: Bubble graphs to compare retention and separation values for the chiral analytes in Table S2 depending on their structural features. Large bubbles indicate large values for each molecular descriptor.

Table 1 system constants and statistics for the 2 columns tested
obtained with Eq.(1), with or without additive in the mobile phase
n is the number of solutes finally retained in the multiple linear regression;
 R^2_{adj} is the adjusted determination coefficient; *SE* in the standard error in the estimate

Column	c	e	s	a	b	v	d⁻	d⁺	f	g	n	R²_{adj}	SE
Whelk-O1	-0.640	0.598	0.184	0.361	0.413	-0.039	-0.137	0.258	0.000	-0.190	212	0.856	0.280
	<i>0.167</i>	<i>0.048</i>	<i>0.056</i>	<i>0.046</i>	<i>0.068</i>	<i>0.058</i>	<i>0.047</i>	<i>0.053</i>	<i>0.036</i>	<i>0.071</i>			
ReproSil Chiral-NR	-0.413	0.462	0.159	0.307	0.284	-0.024	-0.081	0.259	-0.021	-0.195	200	0.854	0.142
	<i>0.136</i>	<i>0.040</i>	<i>0.045</i>	<i>0.037</i>	<i>0.055</i>	<i>0.047</i>	<i>0.038</i>	<i>0.049</i>	<i>0.030</i>	<i>0.058</i>			