



HAL
open science

Analytical and preparative enantioseparations in supercritical fluid chromatography using different brands of immobilized cellulose tris (3,5-dichlorophenylcarbamate) columns: Some differences

Anca-Elena Dascalu, David Speybrouck, Muriel Billamboz, David Corens, Alina Ghinet, Emmanuelle Lipka

► To cite this version:

Anca-Elena Dascalu, David Speybrouck, Muriel Billamboz, David Corens, Alina Ghinet, et al.. Analytical and preparative enantioseparations in supercritical fluid chromatography using different brands of immobilized cellulose tris (3,5-dichlorophenylcarbamate) columns: Some differences. *Journal of Chromatography A*, 2020, 1622, pp.461125 -. 10.1016/j.chroma.2020.461125 . hal-03490204

HAL Id: hal-03490204

<https://hal.science/hal-03490204v1>

Submitted on 3 Jun 2022

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 **Analytical and preparative enantioseparations in supercritical fluid chromatography**
2 **using different brands of immobilized cellulose tris (3,5-dichlorophenylcarbamate)**
3 **columns: some differences.**

4
5 Anca-Elena DASCALU^{1,2,3,4}, David SPEYBROUCK⁵, Muriel BILLAMBOZ^{1,2}, David
6 CORENS⁶, Alina GHINET^{1,2,4}, Emmanuelle LIPKA^{1,3*}

7
8 ¹ U1167 Inserm RID-AGE, Université de Lille, F-59000 Lille

9 ² Yncréa Hauts-de-France, Laboratory of Sustainable Chemistry and Health, Health &
10 Environment Department, Team Sustainable Chemistry, Ecole des Hautes Etudes d'Ingénieur
11 (HEI), UCLille, 13 rue de Toul, F-59046 Lille, France

12 ³ UFR Pharmacie, Laboratoire de Chimie Analytique, BP 83, F-59006 Lille, France

13 ⁴ Alexandru Ioan Cuza' University of Iasi, Faculty of Chemistry, Bd. Carol I nr. 11, 700506

14 ⁵ Discovery Sciences, Janssen Research and Development, Campus de Maigremont, F-27106
15 Val de Reuil cedex France

16 ⁶ Discovery Sciences, Janssen Research and Development, Janssen Pharmaceutica NV,
17 Turnhoutseweg 30, 2340 Beerse, Belgium

18
19 * Corresponding author : Dr Emmanuelle LIPKA

20 Laboratoire de Chimie Analytique - Faculté de Pharmacie de Lille

21 BP 83 – 59006 Lille Cedex - FRANCE

22 E-mail : emmanuelle.lipka@univ-lille.fr

23
24 Keywords: Cellulose *tris* (3,5-dichlorophenylcarbamate); Enantiomers; Loadability;

25 **Manufacturers**; Supercritical fluid chromatography.

26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51

ABSTRACT

The aim of this study was to determine the impact of the origin and the manufacturing processes of the chiral stationary phases (CSPs) on their chromatographic behaviors. Hence, four chiral stationary phases based on immobilized tris (3,5-dichlorophenylcarbamate) derivative of cellulose supplied by four different manufacturers were evaluated. A set of twenty-nine compounds, including commercially available and in-house synthesized compounds, with a broad range of lipophilicity and polarity was chosen. Three main parameters were evaluated on all stationary phases: retention factor, selectivity and loading capacity. This work highlighted that the retention factor strongly varied according to the manufacturer. Regardless of the characteristic of the tested compounds *i.e* neutral, acidic or basic, there was a trend in retention ability of the four chiral stationary phases: retention was increasing from CHIRAL ART Cellulose-SC, REFLECT I-Cellulose C, Chiralpak IC to Lux i-Cellulose-5. On the contrary, selectivity did not follow the same trend as retention. The difference in selectivity between each column towards one compound was quite low while the difference in resolution depended on the nature of the compounds investigated and was significant in certain cases. Finally, the four different columns presented similar and high loading capacity.

52 1. Introduction

53 In pharmaceutical industry, the separation of enantiomers is an ever-growing field as
54 enantiomers of the active substance can present different activities and toxicities, having a
55 great impact on the processes of drug discovery and development. Consequently, in the last
56 decades a strong endorsement could be observed, in the development of enantioselective
57 syntheses and the enantioselective separation methods of new drugs.

58 The most important enantioseparation techniques are High-Performance Liquid
59 Chromatography (HPLC) and Supercritical Fluid Chromatography (SFC), which has taken
60 over the field. Chiral SFC has been providing for almost 30 years an efficient pathway to
61 check the chiral purity of compounds, but also to provide enantiomerically pure materials for
62 synthesis, active pharmaceutical ingredients and drugs [1-5].

63 The selection of an appropriate chiral stationary phase (CSP) represents an important step in
64 the development of most chromatographic methods. There is a large range of CSPs, utilized
65 for chiral resolution including polysaccharides. Polysaccharides (cellulose and amylose)
66 derived chiral stationary phases have proved their usefulness as chiral selector in HPLC as
67 well as in SFC, and a wide range of enantiomeric compounds have been successfully
68 separated on these CSPs. These coated polysaccharide-based CSPs are the most popular
69 choice firstly due to their large loadability (with saturation capacity ranged between 7.5 to
70 more than 100 mg/g CSP) which is extremely important for preparative scale chromatography
71 and secondly because this coated polysaccharides technology that run out of patent, has
72 caused a revival of activity from many different manufacturers. Non-chlorinated
73 polysaccharide-based CSPs have successfully resolved a vast majority of chiral compounds in
74 a broad variety of structural classes [6-8], providing small and large quantities of
75 enantiomerically pure active pharmaceutical ingredients for *in-vivo* and toxicological studies
76 [9-10]. However, these phases have one major disadvantage: they are more or less soluble in

77 many organic solvents (tetrahydrofuran or chlorinated solvents, amongst others) thus reducing
78 the choice of organic modifier. However, drawbacks, such as the limited resistance of these
79 coated phases, have been overcome by immobilizing the polysaccharide derivatives, thus
80 allowing a greater choice of co-solvent. The immobilized versions of the existing coated
81 phases are of the utmost importance for the preparative scale for two reasons: i) on the one
82 hand, new co-solvents can improve resolution through improved efficiency, and ii) on the
83 other hand, can in particular cases considerably improve the solubility of the sample, which in
84 turn allows to increase the productivity [11-13].

85 More recently introduced chlorinated polysaccharide CSPs (either coated or immobilized) can
86 provide additional selectivity to the non-chlorinated CSPs. Chankvetadze's studies [14-16]
87 and Zhang's work [17] on the effect of various groups on the phenyl moiety of different
88 carbamate CSPs, have shown a link between the structural properties and the separation
89 performance of the CSPs. The inductive effect of the chlorine atoms, strong electron
90 withdrawing groups (EWG), can enhance the tendency of the hydrogen atoms to form
91 hydrogen bonds, which can modify the chemical properties of the CSP, and improve the
92 chiral recognition. Since separation performance of a chromatographic column strongly
93 depends on the properties of the stationary phase and therefore results from the manufacturing
94 process, it is important to have reliable information for an adequate selection of the brand.

95 The aim of our study was a practical comparison of cellulose tris (3,5-
96 dichlorophenylcarbamate) columns purchased from four different manufacturers to evaluate
97 their chromatographic performances. For this comparison, a set of different chromatographic
98 parameters for different racemates were evaluated. In this study both commercially available
99 and in-house synthesized compounds, containing one chiral center were used. Preparative
100 chromatography has emerged as an asset in the synthesis of preclinical drug candidates and
101 SFC has become a widely accepted technique for the small-scale preparation (hundreds of

102 milligrams) of pure enantiomers of novel drug candidates needed for preclinical and early
103 clinical studies [18]. Therefore, the loadability of each column was also studied, keeping in
104 mind that this work is not intended to explain the differences observed because the
105 manufacturing process is confidential and might be the same anyway, but rather to give
106 practical information to users in order to save time in method development.

107 2. Material and methods

108 2.1 Chemicals

109 Methanol (MeOH), ethanol (EtOH), propan-2-ol (iPrOH) and acetonitrile (ACN) were of
110 HPLC grade and were purchased from Carlo-Erba Reagents (Val-de-Reuil, France). Carbon
111 dioxide (CO₂) of 99.995% purity was purchased from Linde (Saint Priest, France).
112 Pharmaceutical compounds and triethylamine (TEA) were purchased from Sigma-Aldrich
113 (Saint-Quentin Fallavier, France) and compounds 21-29 (proprietary structure, Figure 1) were
114 synthesized internally.

115 2.2 Sample solutions

116 For analytical screening, solutions of samples were prepared in methanol at 1 mg/mL. For the
117 loading study, solutions of samples were prepared in methanol at 10 mg/mL (excepted 1
118 mg/mL for lenalidomide). The solutions were always degassed by an ultrasonic bath and
119 filtered through a 0.45 µm PTFE syringe-filter (15 mm diameter) before use.

120 2.3 Supercritical Fluid Chromatography Instrument

121 2.3.1 Stationary phases

122 Four chiral analytical columns of cellulose *tris* (3,5-dichlorophenylcarbamate) from different
123 suppliers were used for this study.

124 Chiralpak IC was purchased from Chiral Technologies Europe (Illkirch, France), Lux i-
125 Cellulose-5 from Phenomenex® (Le Pecq, France), CHIRAL ART Cellulose-SC from YMC
126 Europe (Cluzeau Info Labo, Sainte-Foy-La-Grande, France) and the REFLECT I-Cellulose C

127 from Regis Technologies (Thermo Fisher Scientific, Villebon-Courtaboeuf, France). All
128 columns had dimensions of 250x4.6 mm with 5 μ m particle size. The polysaccharide was
129 immobilized on a silica-gel support.

130 2.3.2. Chromatographic system and conditions

131 The chromatographic system used was an SFC-PICLAB hybrid 10-20 apparatus (PIC
132 Solution, Avignon, France) equipped with an autosampler comprising a 48-vials plate (model
133 Alias, Emmen, Netherlands), three model 40 P pumps: two for CO₂ and a third for the
134 modifier (Knauer, Berlin, Germany), a column oven with a Valco ten-position column
135 selection valve, and a Valco six-position solvent switching valve. The system was also
136 composed of a Smartline 2600 diode array detector (DAD) (Knauer, Berlin, Germany).
137 Detection wavelength was set at 254 nm. The system was controlled, and the data was
138 acquired with the SFC PicLab Analytic Online v.3.1.2 software (PIC Solution, Avignon,
139 France). The volume injected was 20 μ L. For the loading experiment, the injected volumes
140 were 50, 100, 250, 485 μ L, respectively. During the separation optimization, the used flow
141 rate was 4 mL/min (excepted for acebutolol (cpd 19) for which the flow rate was 6 mL/min)
142 and the mobile phase was always CO₂-modifier mixture with 15% of methanol as modifier.
143 The exceptions being lenalidomide, compound 25, compound 26 and compound 27 for which
144 the modifier percentage was 30% instead of 15%. For compounds 5, 11, 12, 15, 16, 17 and
145 18, 5% of methanol was also tested to avoid the co-elution with the solvent peak in order to be
146 able to evaluate the selectivity. For acebutolol (cpd 19) and 1-(1-naphthyl)ethylamine (cpd
147 20), 0.3% of triethylamine (TEA) was added to the co-solvent. All analyses were run in
148 isocratic mode. The column oven temperature was 40 °C and the outlet pressure was
149 maintained at 150 bar for all the experiments.

150 Retention times were average values of two replicate determinations. The data was processed
151 by SFC New Data Manager V.1.8.0 software (Pic Solution, Avignon, France).

152 3. Results and discussion

153 In this work, four chiral stationary phases based on immobilized tris (3,5-
154 dichlorophenylcarbamate) derivative of cellulose, coming from four different manufacturers,
155 were evaluated and cited along the manuscript as four columns for the sake of clarity. For all
156 of them, the polysaccharide was immobilized to the surface of a silica matrix. This work
157 notably investigated the practical impact of the different brands on retention, selectivity,
158 efficiency, resolution and loading capacity. Figure 1 shows the selected compounds with a
159 broad range of lipophilicity and polar surface area. All the chromatographic parameters are
160 summarized in Table 1. The direct comparison of the chromatographic parameters was
161 enabled by i) the supercritical conditions used (high percentage of co-solvent and high back
162 pressure) permitting to limit the compressibility of the mobile phase, thus limiting density
163 variation along the column, and therefore the polarity and ii) the similar dimensions of the
164 four columns. The inlet pressure was noticed for each column leading to a pressure drop of 37
165 bar on Lux i-Cellulose-5, of 25 bar on Chiralpak IC, of 26 bar on REFLECT I-Cellulose C
166 and of 24 bar on CHIRAL ART Cellulose-SC column (these values will be discussed later in
167 the manuscript).

168 3.1 Comparison of retention factors

169 First of all, the graph displayed in Figure 2 showed that the retention factors of the second
170 enantiomer eluted (k_2) varied a lot according to the column supplier, e.g. the retention factor
171 of omeprazole (compound 1) ranged from 15.9 on CHIRAL ART Cellulose-SC provided by
172 YMC to 28.3 on Lux i-Cellulose-5 provided by Phenomenex with intermediate values for
173 both Chiralpak IC and REFLECT I-Cellulose C from Daicel and Regis, respectively.

174 Remarkably, the most retentive column was Lux i-Cellulose-5 from Phenomenex for all the
175 selected compounds (except for compounds 18 and 28) regardless of the nature of the analyte,
176 neutral, basic or acid. In addition, the retentiveness of the four columns in decreasing order

177 was always the same going from Lux i-Cellulose-5 (pink plots), Chiralpak IC (orange plots),
178 REFLECT I-Cellulose C (blue plots) to CHIRAL ART Cellulose-SC (green plots). Meaning
179 that the retention factors and retention times as well, are higher on the former column and
180 lower on the latter one. This behavior is exemplified for acebutolol (compound 19) in Figure
181 S1 (in Supplementary Information). In enantioselective chromatography, retention can result
182 from either achiral interactions, or chiral interactions, or both. The main adsorbing site of the
183 cellulose tris (3,5-dichlorophenylcarbamate) derivative CSP is the polar carbamate group
184 which can interact with analytes through hydrogen bonds and dipole-dipole interactions [19].
185 The hydrogen bonds are involved in both non-enantioselective and enantioselective
186 interactions. For this series of compounds, the difference of retention of the second
187 enantiomer eluted on the four different columns compared may be the consequence of a
188 difference in achiral or chiral interactions. However, first, a similar trend was observed for the
189 retention of the first enantiomer eluted of each racemate (k_1) as for the second eluted
190 enantiomer (k_2). This means that a difference of retention for the same compound was
191 observed between the four stationary phases whatever the enantiomers analyzed. Secondly,
192 the graph on Figure 3, depicting the k_2 versus the selectivity (α) for all the compounds,
193 showed that there was no link between retention and selectivity. This observation allowed to
194 conclude that the difference in retention capacity of the CSPs was more linked to achiral
195 interactions than to chiral interactions and confirmed that retention was generally not
196 correlated with enantio-resolution because strong interactions may occur between analytes
197 and CSPs while these interactions do not necessarily contribute to the enantioselectivity. The
198 difference of retentions between the four columns may be the result of the difference in
199 manufacturing process used by the supplier. Indeed, Khater *et al.* [20] have shown in an
200 article dedicated to chlorinated polysaccharide stationary phases that the retention of the latter
201 depended obviously on the nature of the polysaccharide and the type of derivatizing group

202 attached to the glucose moieties of the polysaccharide, but also depended on the
203 manufacturer. Indeed, two stationary phases with the same chiral selector can exhibit different
204 retentions towards solutes. According to Khater *et al.*, the difference linked to the
205 manufacturers was probably due to deviations in the manufacturing procedure between the
206 different suppliers. In our study, significant differences of retentions were observed between
207 the four columns, while the polysaccharide and the side chains are the same for all the CSPs
208 leading to the same conclusion about the manufacturers. One of the explanations could be
209 linked to the process used to immobilize the polysaccharide on the support. Indeed, several
210 methods were used to immobilize the polysaccharide derivatives on silica gel: (i) Okamoto *et*
211 *al.* [21-22] developed a method using diphenyl diisocyanate as a cross linking agent and (ii)
212 the covalent attachment of cellulose derivative via an epoxide moiety [23]. Another
213 possibility (iii) was the process developed by Francotte consisting in exposing the coated
214 phases to photochemical [24] or thermal treatment. A last way (iv) which was a
215 polymerization of a mixed 10-undecenoyl-3,5-dimethylphenylaminocarbonyl derivative of
216 cellulose on the matrix was depicted by Minguillon *et al.* [25]. The process of immobilization
217 of the four columns evaluated in this work is confidential or patented. However, it is obvious
218 that the immobilization procedure used by the different suppliers could vary with an impact
219 on the quantity of chiral selector immobilized (carbon load percentage). In the same manner,
220 the three-dimensional structure of the polysaccharide after immobilization can vary, resulting
221 in some differences on the retention. In addition, as explained by Khater *et al.* [26], the
222 immobilization process led to the presence of residual hydroxyl groups on the polysaccharide
223 and the percentage of hydroxyl groups may be different according to the CSPs with an impact
224 on the retention. Finally, the nature of the silica gel may vary with a diversity of pore sizes,
225 surface area, with the use of bare silica or aminopropyl silica or even different pre-treatment
226 of the support leading to a diversity of physical properties of the stationary phases. All these

227 specificities of the four columns **resulted** in different retention capacities. In conclusion,
228 although the chiral selector **was** the same in all the four columns, a large difference in the
229 ability to retain the compounds **was** observed.

230 3.2 Comparison of selectivity

231 Just like the retention factors were different on the columns for each of the tested compounds,
232 the trend **in selectivity was** also **diverse**.

233 Firstly, unlike the retention factor, which varied a lot for this set of compounds, the difference
234 of selectivity between each column **towards a compound was** quite low as exemplified in
235 **Figure S2** (in Supplementary Information) for compound **3** (lenalidomide) for which the
236 selectivity values **were** equal to 2.44, 2.12, 2.39 and 2.31 (Table 1), respectively for Chiralpak
237 IC, Lux i-Cellulose-5, CHIRAL ART Cellulose SC and REFLECT I-Cellulose C. **Those four**
238 **values of selectivity represented a relative standard deviation of 6.07% which was the highest**
239 **calculated among the 29 compounds**.

240 Secondly, the column providing the highest selectivity **was** not the same towards all the
241 compounds. For the compound **20**, the best separation factor was obtained **within Chiralpak**
242 **IC**. The CHIRAL ART Cellulose-SC column **provided** the best selectivity for the compound
243 **14**, while the best separations of the compounds **4** and **25** were obtained on REFLECT I-
244 Cellulose C and Lux i-Cellulose-5 respectively. Nevertheless, as illustrated in **Figure S3a** (in
245 Supplementary Information), **Chiralpak IC gave** the best selectivity for **29.2%** of the **tested**
246 compounds and the highest number of compounds separated ($\alpha > 1$) **was** obtained **on the**
247 **CHIRAL ART Cellulose-SC and REFLECT I-Cellulose C columns** with **58.7%** of separation
248 (**figure S3b**, in Supplementary Information). **Remarkably**, for eight racemates no separation
249 was achieved **on any of the four studied** columns. The chiral recognition ability of the
250 columns **was** different towards compounds **28** and **29**. Indeed, the enantiomers of the
251 compound **28 were** separated both on CHIRAL ART Cellulose-SC and REFLECT I-Cellulose

252 C, while no separation was observed on Chiralpak IC and Lux i-Cellulose 5 in the
253 implemented conditions (Figure S4a, in Supplementary Information). Identically, for the
254 compound **29**, the enantiomers were not separated on Chiralpak IC while they were partially
255 separated on the three other columns in the implemented conditions (Figure S4b, in
256 Supplementary Information).

257 Regarding the fifteen compounds separated on the four columns, an average separation factor
258 was calculated for each column to give a higher recognition ability for Chiralpak IC for those
259 compounds. Hence, despite the same chiral selector, some differences were observed between
260 these four different columns. Indeed, the conditions used to immobilize the chiral selector are
261 probably different for the four columns with an impact on the chiral recognition. Like the
262 coating conditions on silica gel significantly influence their chiral recognition ability, the
263 process used to immobilize the polysaccharide may impact the chiral recognition too [27].
264 The utilization of different processes of immobilization may change the polymer
265 configuration and/or the supramolecular structure of the polysaccharide. The high order
266 structure of the polysaccharide is probably more or less maintained according to the
267 manufacturer with an impact on the recognition ability. In conclusion, most of the time
268 selectivity followed the same trend from one column to another, except for compounds **28** and
269 **29**.

270 3.3 Comparison of plate number and resolution

271 The efficiency and resolution values were reported in Table 1 (Supplementary Information).
272 For a fair comparison, only the 14 fully baseline resolved compounds were chosen. Mainly
273 because efficiency can be wrongly evaluated for non-separated peak (whose peak width can
274 be falsely increased by a beginning of separation of both enantiomers).
275 The plate number was slightly different between the four columns for every compound.
276 However, these discrepancies were not deeply studied and no conclusion was drawn from this

277 observation because the inlet pressure measured for every column was not the same (as
278 depicted before) resulting in a difference of diffusion coefficient in the mobile phase with a
279 potential impact on the efficiency.

280 Like the selectivity, the difference of resolution between each column towards a compound
281 depended on the compound itself as explained by Figure 4. Some discrepancies were
282 observed as the relative standard deviation were calculated for the four resolution values
283 obtained on the four columns for each compound. For instance, the difference between the
284 four columns was equal to 6.24% for compound **7** (indoprofen) which was quite low, meaning
285 that the four results obtained on different manufacturer columns were similar. But for some
286 compound this difference reached 17.56% for compound **1** (omeprazole) with resolution
287 values equal to 10.27; 6.64; 9.01 and 9.39 and even 19.78% for compound **2** (thalidomide)
288 with values equal to 4.39; 2.82; 4.52; 3.89 which was significant. For each compound, the
289 four columns were classified either first or second position (out of four) depending on the
290 resolution obtained. Among the fourteen compounds, the Chiralpak IC appeared seven times
291 in first position and four times in second position, allowing the best resolution for eleven
292 compounds out of fourteen. This column was followed by the CHIRAL ART Cellulose SC
293 column with four first positions and three second positions. But with watching the Figure 4
294 and Table 1, there were no drastic differences between columns, at two exceptions. For
295 compound **28** a full resolution occurred for two columns (CHIRAL ART Cellulose SC and
296 REFLECT I-Cellulose C) while no enantiomeric separation was observed nor on Lux i-
297 Cellulose-5 neither on Chiralpak IC in the implemented conditions (Figure S4, in
298 Supplementary Information). And for compound **29** for which the enantiomers were not
299 separated on Chiralpak IC while they were partially separated on the three other columns in
300 the implemented conditions (Figure S4b, in Supplementary Information).

301 Among the 29 compounds studied in this work, high resolutions were achieved on the four
302 columns for compounds **1** (omeprazole); **2** (thalidomide); **4** (warfarin); **14** (tropicamide); **20**
303 (1-(1-Naphthyl)ethylamine)) and in-house compounds **21** and **22**, under 15% MeOH and for
304 compounds **3** (lenalidomide) and in-house compound **26** under 30% MeOH, with various
305 retention times.

306 3.4 Comparison of the loading capacity

307 The results displayed in the three previous sections showed a significant difference of
308 retention, differences of resolution and slight difference of selectivity between the four
309 columns for the tested compounds. In addition to retention, selectivity and resolution, loading
310 capacity can be evaluated because it is a significant parameter in preparative chromatography.
311 Indeed, the loading capacity of the stationary phase will have an impact on the productivity of
312 a separation [13]. Thus, the impact of increasing the amount injected on the four columns was
313 assessed. Two methods can be used to overload a sample on a column: volume overload or
314 mass overload. For volume overload, a feed solution was prepared by dissolving the
315 compound with a defined concentration and then increasing volumes of the solution were
316 injected until the peaks touched at the base (touching band) before resolution will be lost.
317 Sometimes poor peak shape was obtained under high volume of injection. For mass overload,
318 the sample volume was kept constant while the mass of compounds dissolved in this volume
319 was increased progressively. Usually volume overload is used in preparative chiral separation
320 in discovery because the method development is faster, explaining why this process was
321 chosen in this work. Five compounds were selected, based on i) their high-resolution result
322 under 15% MeOH as co-solvent for all of them (excepted 30% for lenalidomide) and ii) on
323 shorter retention times (when similar resolution values were observed). Therefore
324 omeprazole, warfarin, lenalidomide, tropicamide and the in-house compound **22** were
325 selected.

326 To illustrate this loading capacity linked to the baseline return (or touching band), **the**
327 **following** representations were chosen: i) overloaded chromatograms and ii) the plot of the
328 separation criterion (S) in function of the amount injected.

329 Firstly, considering the overloaded chromatograms, they **were** represented for the in-house
330 compound **22** (**Figure 5**) and for omeprazole (**Figure 6**). As shown in **Figures 5** and **6**,
331 increasing volume of feed solution (10 mg/mL) **impacted** the peak shape. This modification of
332 the peak shape may lead to a reduction of the resolution with, for some compounds, the
333 observation of one broad peak instead of two for the larger injected volume. For the in-house
334 compound **22**, a loss of baseline separation **was** observed for 2.50 mg injected (250 μ L of feed
335 solution) on the four columns. Remarkably, for omeprazole, the retention times at the peak
336 ends **were** constant whatever the injection volume, and the retention times at the beginning of
337 the peak **decreased** in proportion to the injection volume. For the analysis of omeprazole
338 (**Figure 6**), a baseline resolution **was** observed for 4.85 mg injected (485 μ L) on Chiralpak IC
339 while a slight overlap of the peaks **was** observed for the three additional columns. However,
340 the phenomenon **was** more the consequence of a lower selectivity on the three columns
341 compared to Chiralpak IC **and** a lower retention on **the CHIRAL ART Cellulose-SC column**
342 than a question of loadability since any significant difference in the peak shapes **was**
343 observed.

344 As the resolution is difficult to determine for broad and asymmetric peak shape, the
345 measurement of the separation criterion (S) was also used to monitor the quality of the
346 separation of two successive peaks according to the injected amount [28]. S is defined as the
347 difference between the retention time at the beginning of the second peak (t_{B2}) and the
348 retention time at the end **of** the first peak (t_{E1}); peaks are baseline resolved if S is positive: $S =$
349 $t_{B2} - t_{E1}$. The graphs for omeprazole and in-house compound **22** on **Figure 7** (and for
350 lenalidomide, tropicamide and warfarin on **Figure S5**, in Supplementary Information) **showed**

351 the same trend for all the compounds **regardless column brand**. The increase in injected
352 volume **caused** a reduction of the separation criterion with no significant difference observed
353 between the four columns.

354 The loading capacity of a stationary phase towards a compound **strongly depends on the**
355 selectivity (α) but is also impacted by the efficiency of the column and the retention factor. In
356 other words, it also depends on the resolution. In addition, the loadability of a stationary phase
357 is a function of the number of accessible interaction sites per mass unit of phase, so is
358 dependent of the density of chiral selectors or carbon load [29]. The **behavior** of the four
359 columns towards increasing injected amount **didn't** differ substantially, leading to the
360 conclusion that the four columns have quite similar density of chiral selectors.

361 **4. Conclusion**

362 The comparison of the different immobilized cellulose *tris* (3,5-dichlorophenylcarbamate)
363 columns *i.e.* Chiralpak IC, Lux i-Cellulose 5, CHIRAL ART Cellulose-SC and REFLECT I-
364 Cellulose C, by use of 29 representative racemic mixtures **showed** some differences. Indeed,
365 the retention factors **were** significantly different with a higher retention on the Lux i-Cellulose
366 5 and with the lowest retention on Chiral ART Cellulose-SC **for all compounds (excepted**
367 **compounds 18 and 28, see Figures 2 and S4a)**. The best selectivity of each of the four
368 columns **depended on the nature of the compounds but differences between selectivity values**
369 **were minor**. Besides, the Chiralpak IC column showed the highest rate of separation.
370 Concerning **the** resolution parameter, **once again**, the best resolution of each of the four
371 columns depended on the compounds **but differences between resolution were quite**
372 **significant for compounds 1 (omeprazole), 2 (thalidomide), 3 (lenalidomide) and in-house 27**
373 **with standard relative deviation comprised between 11% and 20%**. Finally, for the loading
374 capacity, no strong difference **was registered** between the different columns, thus for small-
375 scale separation each of them can be chosen. This study **showed** that despite the same chiral

376 selector, the four stationary phases from different manufacturers were not identical probably
377 due to differences in the manufacturing process such as the immobilisation of the
378 polysaccharide that we do not intend to further explain here, as these processes are
379 confidential. From a practical point of view, if one needs high retention capacity from its
380 column, then choose a Lux i-Cellulose-5, on the contrary if your compounds elute too late,
381 one should opt for CHIRAL ART SC-cellulose. If one has an important amount of racemates
382 to separate then the Chiralpak IC seems the most appropriate column and will certainly lead to
383 a high success rate in terms of selectivity.

384 The comparison of the chromatographic parameters obtained on the four immobilized
385 columns with those obtained on the coated version of this chiral selector would have been
386 interesting to determine the impact of the immobilization. But to be realistic, the coated and
387 the immobilized columns should be provided by the same manufacturer. In addition, as
388 explained by S. Carradori, this coated CSP (named as Sepapak-5 from Sepaserve GmbH
389 affiliated to Phenomenex) is no longer commercially available, due to high solubility of this
390 polysaccharide in mobile phase consisted of *n*-hexane/*i*PrOH mixture [30].

391 5. References

- 392 [1] V. Desfontaine, D. Guillarme, E. Francotte, L. Novakova, Supercritical fluid
393 chromatography in pharmaceutical analysis, *J. Pharm. Biomed. Anal.* 113 (2015) 56–71.
- 394 [2] Y.N. Zhao, G. Woo, S. Thomas, D. Semin, P. Sandra, Rapid method development for
395 chiral separation in drug discovery using sample pooling and supercritical fluid
396 chromatography-mass spectrometry, *J. Chromatogr. A* 1003 (2003) 157–166.
- 397 [3] M. Maftouh, C. Granier-Loyaux, E. Chavana, J. Marini, A. Pradines, Y.
398 VanderHeyden, C. Picard, Screening approach for chiral separation of pharmaceuticals. Part
399 III. Supercritical fluid chromatography for analysis and purification in drug discovery, *J.*
400 *Chromatogr. A* 1088 (2005) 67–81.

- 401 [4] G.F. Pirrone, R.M. Mathew, A.A. Makarov, F. Bernardoni, A. Klapars, R. Hartman, J.
402 Limanto, E.L. Regalado, Supercritical fluid chromatography-photodiode array detection-
403 electrospray ionization mass spectrometry as a framework for impurity fate mapping in the
404 development and manufacture of drug substances, *J. Chromatogr. B* 1080 (2018) 42–49.
- 405 [5] C. White, Integration of supercritical fluid chromatography into drug discovery as a
406 routine support tool: part I: fast chiral screening and purification, *J. Chromatogr. A* 1074
407 (2005) 163–173.
- 408 [6] K. Kalíková, T. Šlechtová, J. Vozka, E. Tesařová, Supercritical fluid chromatography
409 as a tool for enantioselective separation; a review, *Anal. Chim. Acta* 821 (2014) 1–33.
- 410 [7] L. Toribio, J.L. Bernal, M.J. del Nozal, J.J. Jiménez, E.M. Nieto, Applications of the
411 Chiralpak AD and Chiralcel OD chiral columns in the enantiomeric separation of several
412 dioxolane compounds by supercritical fluid chromatography, *J. Chromatogr. A* 921 (2001)
413 305–313.
- 414 [8] J.L. Bernal, L. Toribio, M.J. del Nozal, E.M. Nieto, M.I. Montequi, Separation of
415 antifungal chiral drugs by SFC and HPLC: a comparative study, *J. Biochem. Biophys.*
416 *Methods* 54 (2002) 245–254.
- 417 [9] Y.K. Ye, K.G. Lynam, R.W. Stringham, Effect of amine mobile phase additives on
418 chiral subcritical fluid chromatography using polysaccharide stationary phases, *J.*
419 *Chromatogr. A* 1041 (2004) 211–217.
- 420 [10] D. Wu, L. Leith, B. Balasubramanian, T. Palcic, D. Wang-Iverson, The impact of
421 chiral supercritical chromatography in drug discovery: from analytical to multi gram scale,
422 *Am. Laborat.* 38 (2006) 24–26.
- 423 [11] L. Miller, Evaluation of non-traditional modifiers for analytical and preparative
424 enantioseparations using supercritical fluid chromatography, *J. Chromatogr. A* 1256 (2012)
425 261-266.

- 426 [12] L. Miller, Use of dichloromethane for preparative supercritical fluid chromatographic
427 enantioseparations, *J. Chromatogr. A* 1363 (2014) 323-330.
- 428 [13] D. Speybrouck, E. Lipka, Productivity and solvent waste in supercritical fluid
429 chromatography for preparative chiral separations: a guide for a convenient strategy, *J.*
430 *Chromatogr. A*, 1610 (2020) *In press*.
- 431 [14] B. Chankvetadze, E. Yashima, Y. Okamoto, Chloromethylphenylcarbamate
432 derivatives of cellulose as chiral stationary phases for high-performance liquid
433 chromatography, *J. Chromatogr. A* 670 (1994) 39-49.
- 434 [15] B. Chankvetadze, E. Yashima, Y. Okamoto, Dimethyl-, dichloro- and
435 chloromethylphenylcarbamates of amylose as chiral stationary phases for high-performance
436 liquid chromatography, *J. Chromatogr. A* 694 (1995) 101-109.
- 437 [16] B. Chankvetadze, L. Chankvetadze, S. Sidamonidze, E. Yashima, Y. Okamoto, High
438 performance liquid chromatography enantioseparation of chiral pharmaceuticals using *tris*
439 (chloro-methylphenylcarbamate)s of cellulose, *J. Pharm. Biomed. Anal.* 14 (1996) 1295-1303.
- 440 [17] T. Zhang, D. Nguyen, P. Franco, Y. Isobe, T. Michishita, T. Murakami, Cellulose *tris*
441 (3,5-dichlorophenylcarbamate) immobilized on silica: A novel chiral stationary phase for
442 resolution of enantiomers, *J. Pharm. Biomed. Anal.* 46 (2008) 882-891.
- 443 [18] D. Speybrouck and E. Lipka, Preparative supercritical fluid chromatography: a
444 powerful tool for chiral separations, *J. Chromatogr. A*, 1467 (2016) 33-55.
- 445 [19] N. Enomoto, S. Furukawa, Y. Ogasawara, H. Akano, Y. Kawamura, E. Yashima and
446 Y. Okamoto, Preparation of silica gel-bonded amylose through enzyme-catalyzed
447 polymerization and chiral recognition ability of its phenylcarbamoyl derivative in HPLC,
448 *Anal. Chem.*, 68, (1996) 2798-2804.

449 [20] S. Khater, Y. Zhang and C. West, Insights into chiral recognition mechanism in
450 supercritical fluid chromatography IV. Chlorinated polysaccharide stationary phases, J.
451 Chromatogr. A, 1363 (2014) 294-310.

452 [21] Y. Okamoto and E. Yashima, Polysaccharide derivatives for chromatographic
453 separation of enantiomers, Angew. Chem. Int. Ed. Engl., vol. 37, (1998) 1020-1043.

454 [22] Y. Okamoto, R. Aburatani, S. Miura and K. Hatada, Chiral stationary phases for
455 HPLC: cellulose *tris* (3,5-dimethylphenylcarbamate) and *tris* (3,5-dichlorophenylcarbamate)
456 chemically bonded to silica gel, J. Liquid Chrom., 10 (1987) 1613-1628.

457 [23] B. Chankvetadze, T. Ikai, C. Yamamoto, Y. Okamoto, High-performance liquid
458 chromatographic enantioseparations on monolithic silica columns containing a covalently
459 attached 3,5-dimethylphenylcarbamate derivative of cellulose, J. Chromatogr. A, 1042 (2004)
460 55-60.

461 [24] E. Francotte, Photochemically cross-linked polysaccharide derivatives as supports for
462 the chromatographic separation of enantiomers. Patent WO 96/27615 A1, 12 September 1996.

463 [25] C. Minguillon, P. Franco, L. Oliveros, P. Lopez, Bonded cellulose-derived high-
464 performance liquid chromatography chiral stationary phases I. Influence of the degree of
465 fixation on selectivity, J. Chromatogr. A, 728 (1996) 407-414.

466 [26] S. Khater, Y. Zhang and C. West, Insights into chiral recognition mechanism in
467 supercritical fluid chromatography III. Non-halogenated polysaccharide stationary phases, J.
468 Chromatogr. A, 1363 (2014) 278-294.

469 [27] Y. Okamoto, R. Aburatani and K. Hatada, Chromatographic chiral resolution: XIV.
470 Cellulose tribenzoate derivatives as chiral stationary phases for high-performance liquid
471 chromatography, J. Chromatogr. A, 389 (1987) 95-102.

472 [28] A. Noireau, E. Lemasson, F. Mauge, A. Petit, S. Bertin, P. Hennig, E. Lesselier and C.
473 West, Purification of drug degradation products supported by analytical and preparative
474 supercritical fluid chromatography, *J. Chromatogr. A*, 170 (2019) 40-47.

475 [29] E. Francotte, Chiral stationary phases for preparative enantioselective
476 chromatography, in *Preparative enantioselective chromatography*, Oxford, Blackwell
477 publishing, 2005, pp. 48-77.

478 [30] S. Carradori, D. Secci, C. Faggi and R. Cirilli, A chromatographic study on the
479 exceptional chiral recognition of 2-(benzylsulfinyl)benzamide by an immobilized-type chiral
480 stationary phase based on cellulose *tris*(3,5-dichlorophenylcarbamate), *J. Chromatogr. A*,
481 1531 (2018) 151-156.

482

483

484 **Figure Captions**

485

486 Figure 1: Molecular structures, names and numbers of the analyzed compounds within the
487 SFC study.

488 Figure 2: Retention factor for the 29 compounds with the four CSPs. ● acidic compounds,
489 ■ neutral compounds, ▲ basic compounds.

490 Figure 3: Selectivity vs retention factor for the 29 compounds on the four stationary phases.

491 Figure 4: Resolution for the 29 compounds with the four CSPs. ● acidic compounds, ■
492 neutral compounds, ▲ basic compounds.

493 Figure 5: Chromatograms of the analysis of the in-house compound **22** on the four columns
494 (Chiralpak IC, Lux i-Cellulose 5, CHIRAL ART Cellulose-SC and REFLECT I-Cellulose C).
495 Conditions: mobile phase: CO₂/MeOH 85/15; flow rate: 4 mL/min; temperature: 40 °C; outlet
496 pressure: 150 bar; sample concentration: 10 mg/mL; sample volume: from 20 μL to 250 μL;
497 λ= 254 nm.

498 Figure 6: Chromatograms of the analysis of omeprazole on the four columns (Chiralpak IC,
499 Lux i-Cellulose 5, CHIRAL ART Cellulose-SC and REFLECT I-Cellulose C).
500 Conditions: mobile phase: CO₂/MeOH 85/15; flow rate: 4 mL/min; temperature: 40 °C; outlet
501 pressure: 150 bars; sample concentration: 10 mg/mL; volume injected: from 20 μL to 485 μL;
502 λ= 254 nm.

503 Figure 7: Separation criterion (S) vs injected amount on the four CSPs (Chiralpak IC, Lux i-
504 Cellulose 5, CHIRAL ART Cellulose SC and REFLECT I-Cellulose C) for omeprazole (10
505 mg/mL from 20 μL to 485 μL) and in-house compound **22** (10 mg/mL from 20 μL to 250
506 μL).

507

508

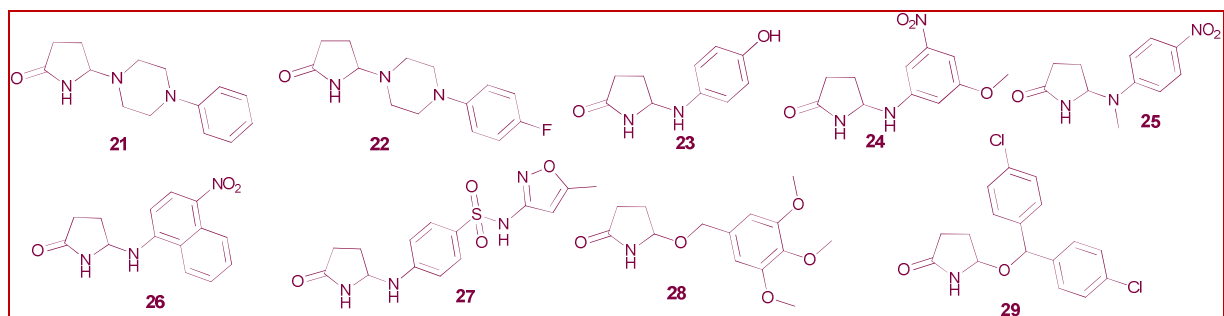
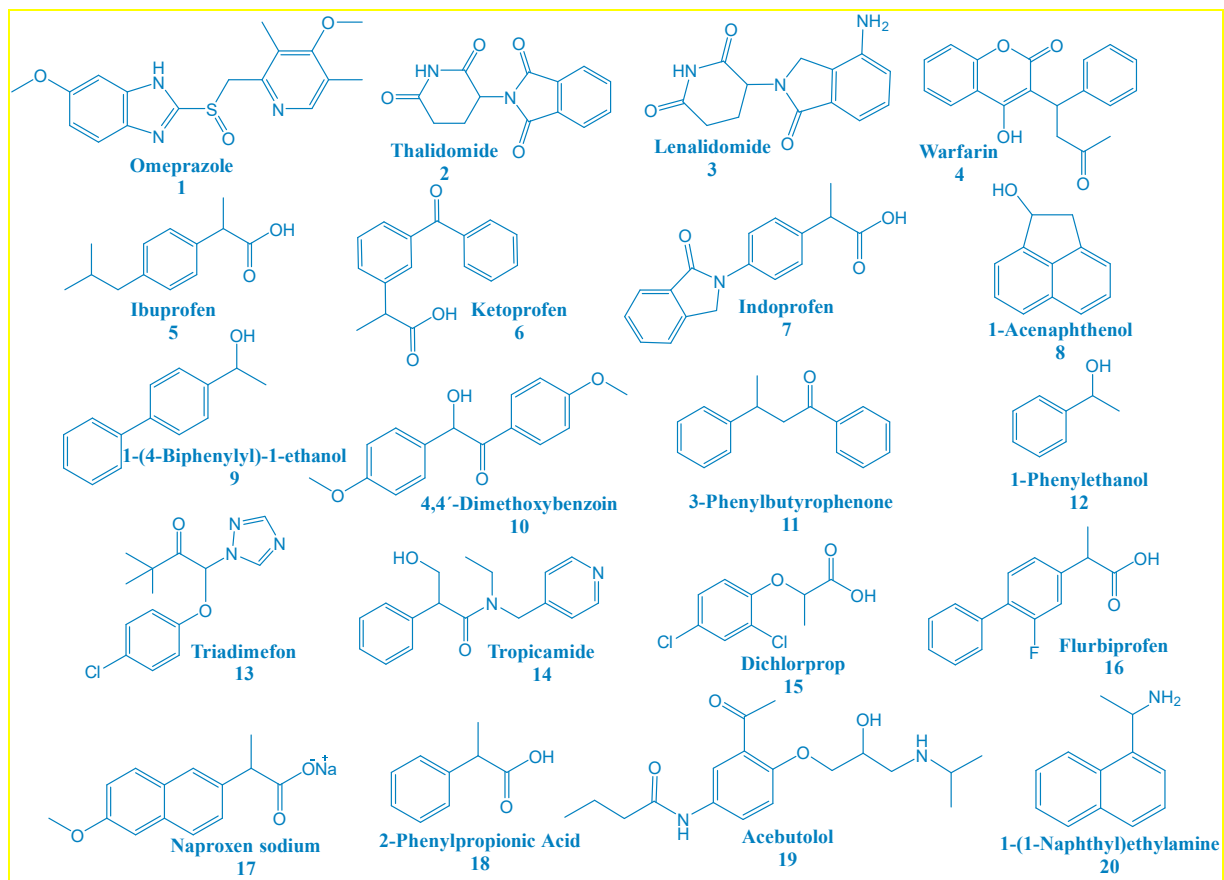


Figure 1

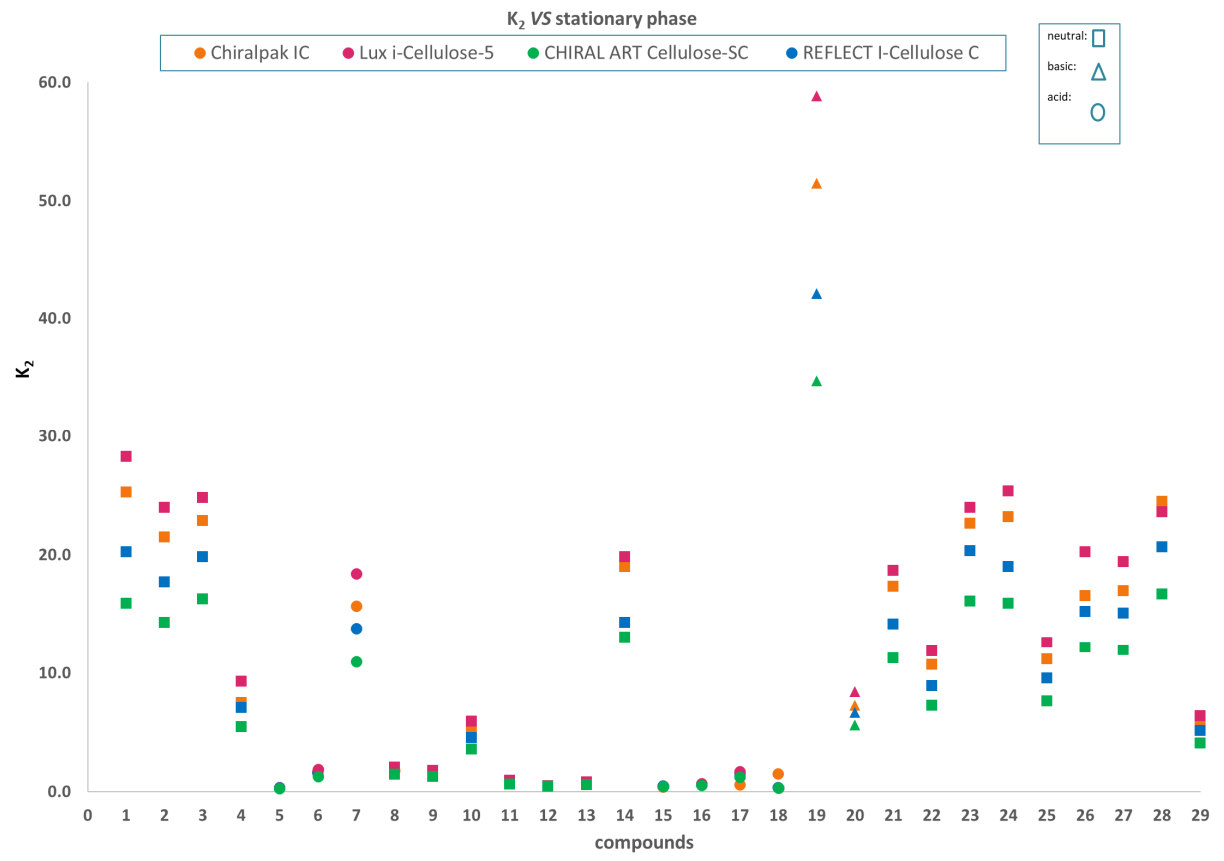


Figure 2

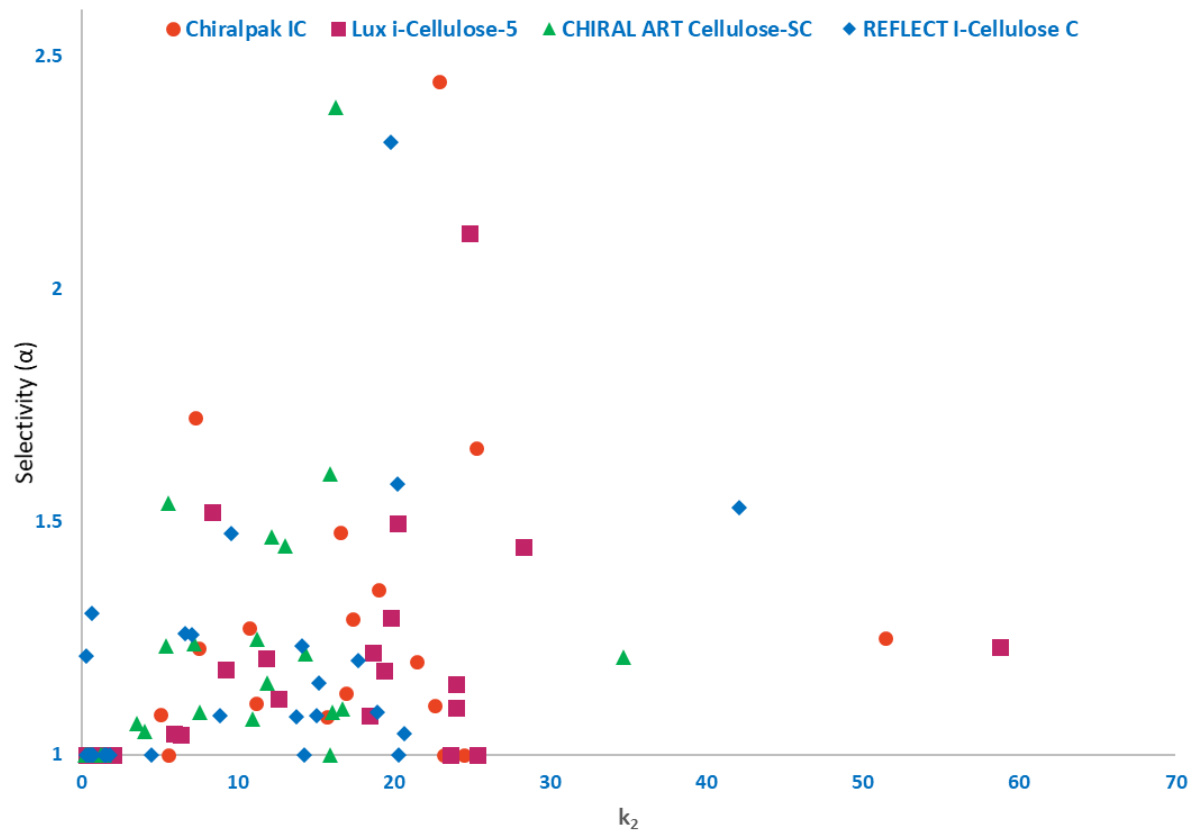


Figure 3

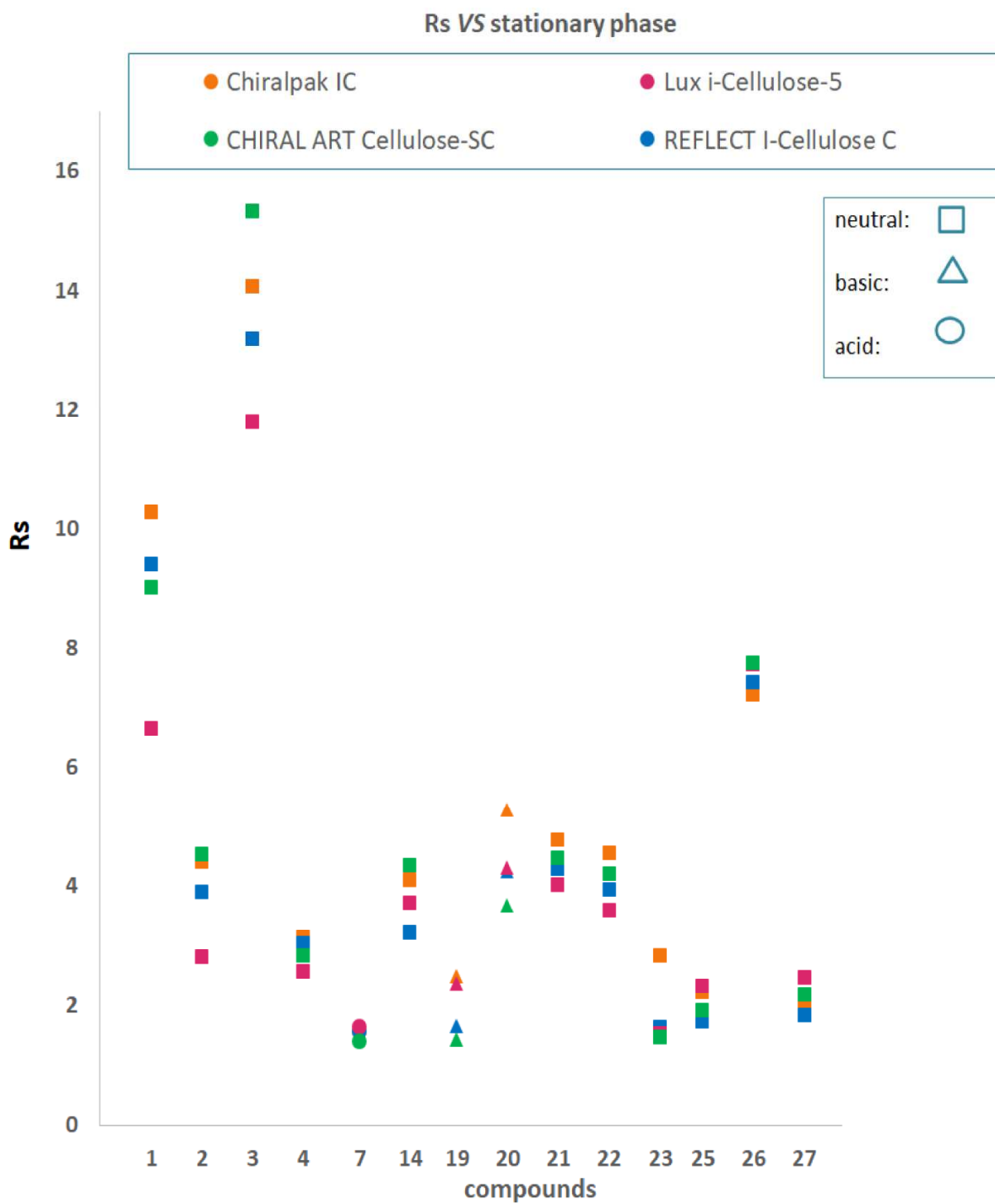
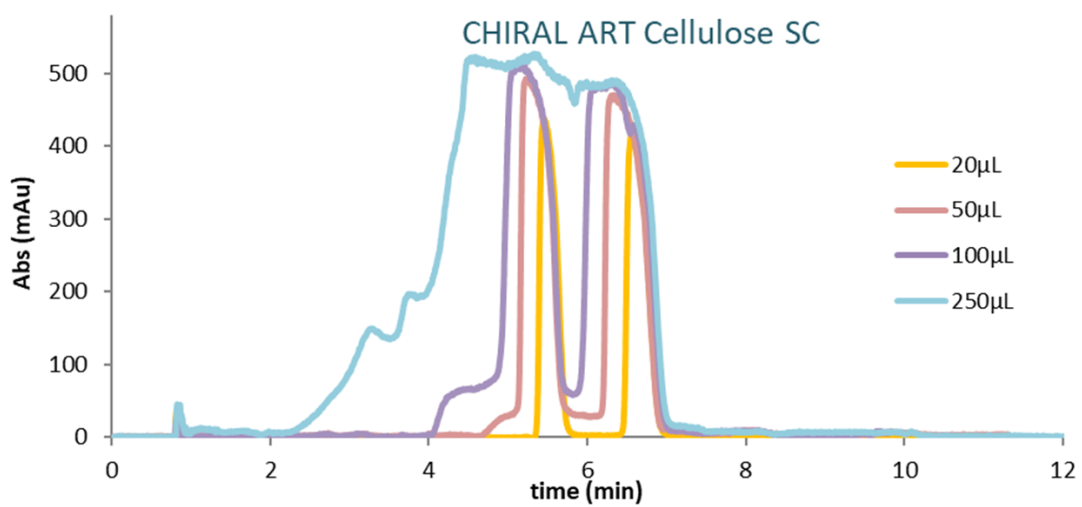
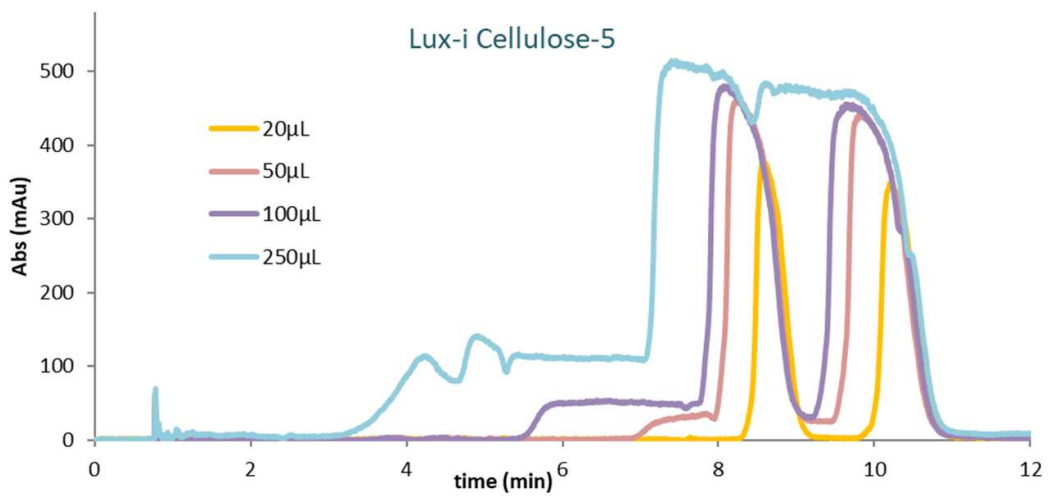
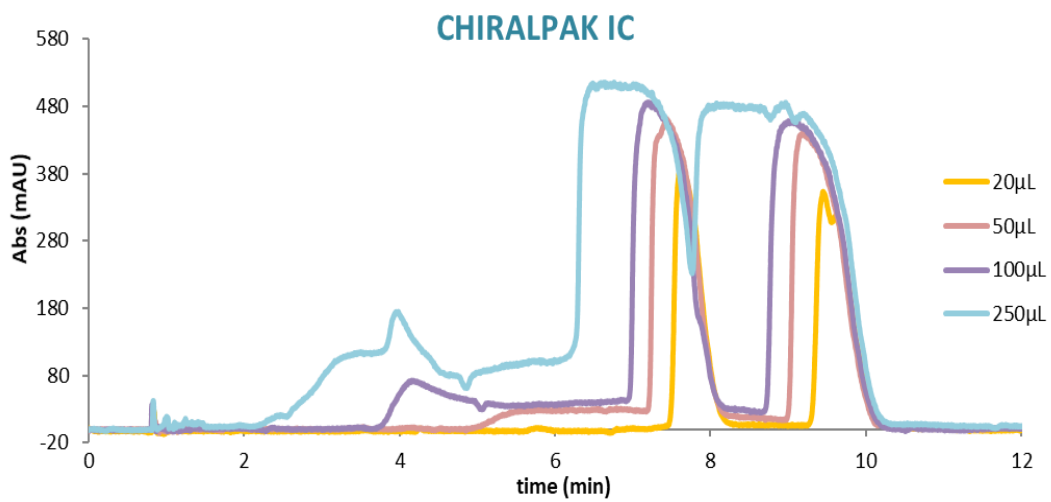


Figure 4



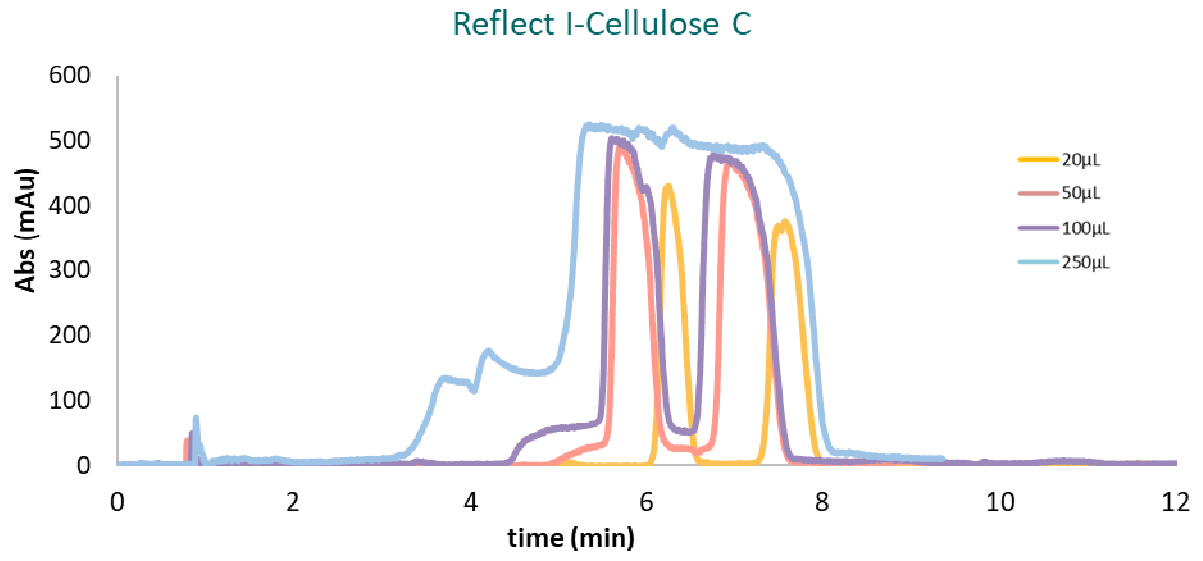
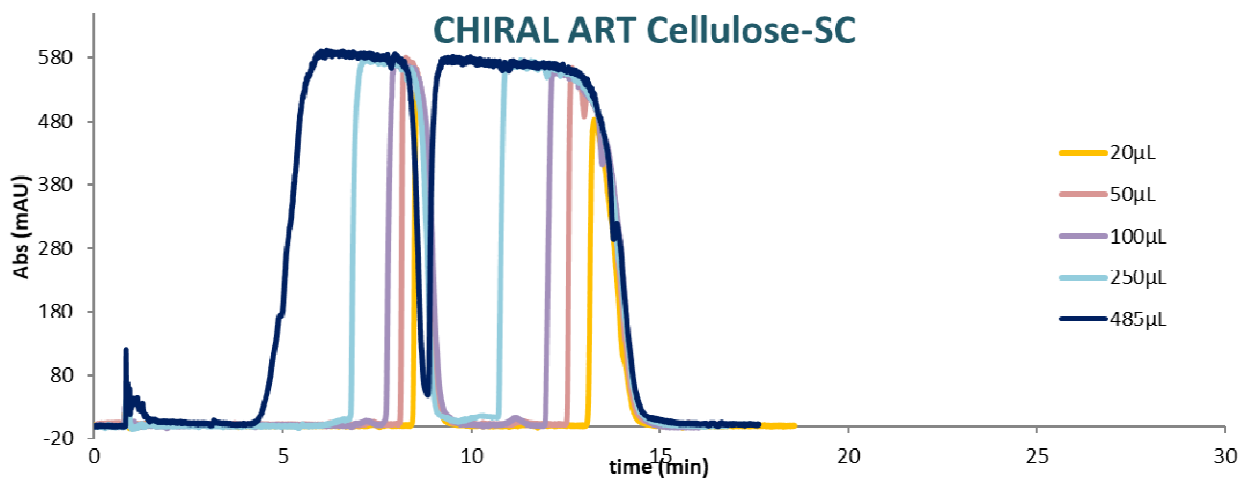
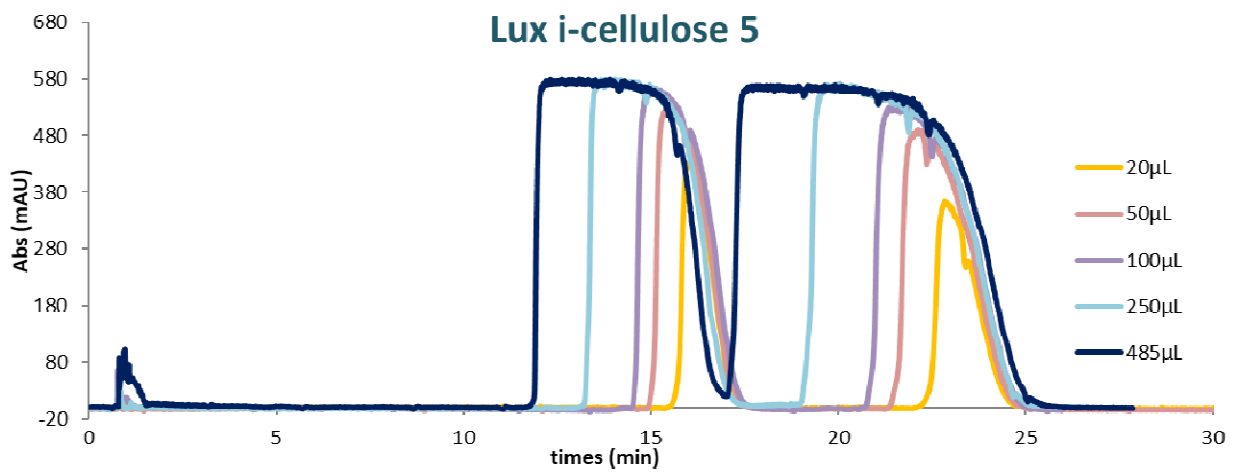
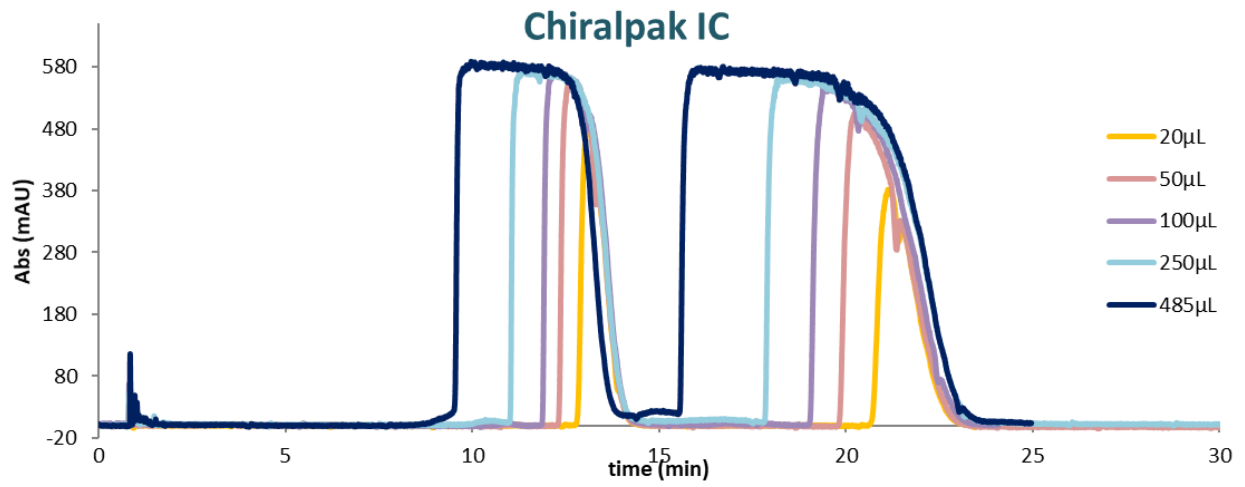


Figure 5



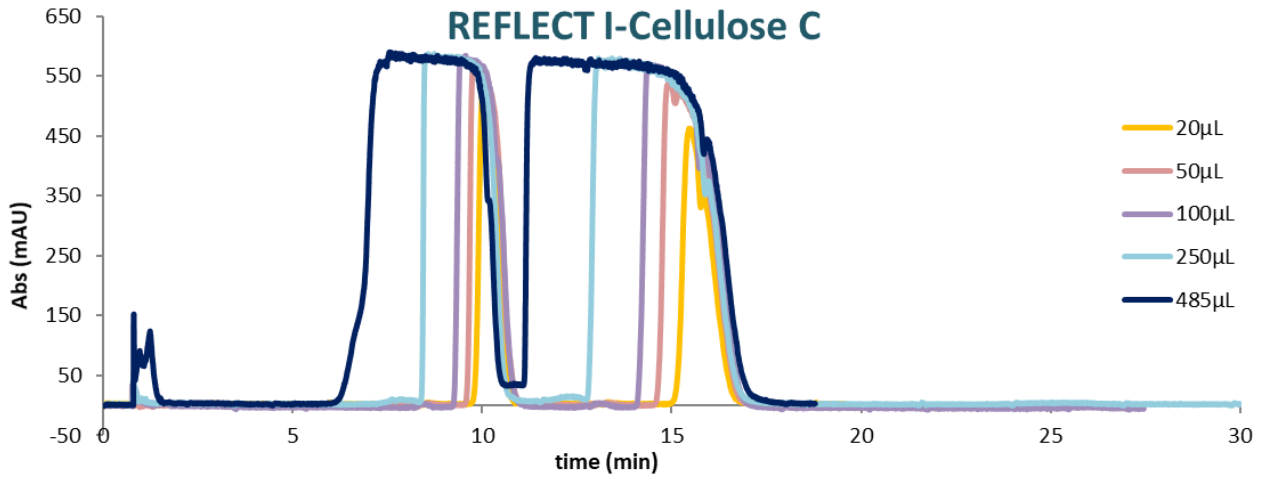


Figure 6

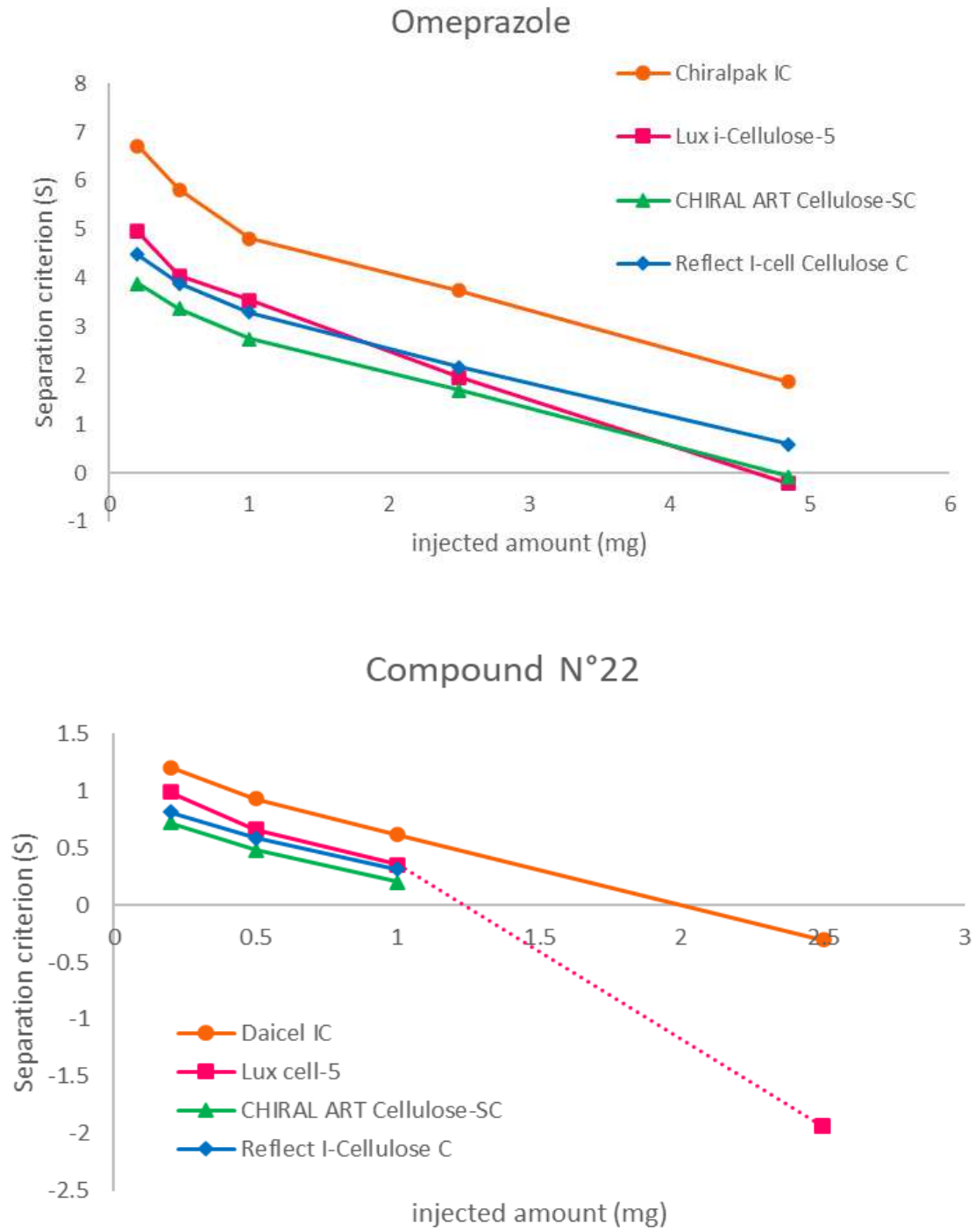


Figure 7