



HAL
open science

Separation of enantiomers of native amino acids with polysaccharide-based chiral columns in supercritical fluid chromatography

Emmanuelle Lipka, Anca-Elena Dascalu, Yasmine Messara, Eka Tsutsqiridze, Tivadar Farkas, Bezhan Chankvetadze

► **To cite this version:**

Emmanuelle Lipka, Anca-Elena Dascalu, Yasmine Messara, Eka Tsutsqiridze, Tivadar Farkas, et al.. Separation of enantiomers of native amino acids with polysaccharide-based chiral columns in supercritical fluid chromatography. *Journal of Chromatography A*, 2019, 1585, p.207 - 212. 10.1016/j.chroma.2018.11.049 . hal-03486024

HAL Id: hal-03486024

<https://hal.science/hal-03486024>

Submitted on 20 Dec 2021

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

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



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Separation of enantiomers of native amino acids with polysaccharide-**
2 **based chiral columns in supercritical fluid chromatography**

3
4 Emmanuelle Lipka^{1*}, Anca-Elena Dascalu^{1,2,3}, Yasmine Messara¹, Eka Tsutsqiridze⁴,
5 Tivadar Farkas⁵, Bezhan Chankvetadze^{4*}

6 ¹⁾ Unité 995 INSERM, Université de Lille, Lille Inflammation Research International
7 Center, Laboratoire de Chimie Analytique-Faculté de Pharmacie de Lille, BP 83 - 59006
8 Lille, France.

9 ²⁾ Hautes Etudes d'Ingénieur (HEI), Yncréa Hauts-de-France, UCLille, Laboratoire de
10 Pharmacochimie, 13 rue de Toul, F-59046 Lille, France.

11 ³⁾ 'Alexandru Ioan Cuza' University of Iasi, Faculty of Chemistry, Bd. Carol I nr. 11,
12 700506 Iasi, Romania.

13 ⁴⁾ Institute of Physical and Analytical Chemistry, School of Exact and Natural Sciences,
14 Tbilisi State University, Chavchavadze Ave 3, 0179 Tbilisi, Georgia.

15 ⁵⁾ Phenomenex Inc., 411 Madrid Ave., Torrance, 90501 CA, USA.

16 *Corresponding Authors:

17 Tel: +33 03 62 28 30 25, E-Mail address: emmanuelle.lipka@univ-lille.fr

18 Tel: +995 555730048, E-mail address: jpba_bezhan@yahoo.com

19 **Abstract**

20 In this short communication preliminary results are reported on the separation of
21 enantiomers of underivatized amino acids on polysaccharide-based chiral columns in
22 sub/super-critical fluid chromatography (SFC).

23 Racemic mixtures of 14 amino acids were used to evaluate the chiral recognition ability
24 of 8 different polysaccharide-based chiral columns with coated or covalently immobilized
25 chiral selectors. Among the studied columns, the one based on cellulose (3,5-
26 dichlorophenylcarbamate) exhibited the most universal chiral recognition ability
27 providing baseline resolution for the enantiomers of 10 studied amino acids. In addition,
28 the effect of mobile phase composition (in particular, of the amount of additive used), as
29 well as the potential contribution of evaporative light scattering detector (ELSD) to extra
30 column band-broadening was evaluated.

31

32 *Keywords:*

33 Separation of enantiomers; Polysaccharide-based chiral stationary phases; Native amino-
34 acids; Mobile phase composition; Extra column band-broadening.

35

36 **1. Introduction**

37 Although polysaccharide derivatives represent the group of most universal chiral
38 selectors for liquid phase separation of enantiomers [1-3], there are certain groups of
39 chiral analytes towards which these chiral selectors have been reported to exhibit
40 inadequate chiral recognition ability. Small nonaromatic carboxylic acids and most native
41 amino acids belong to this category of chiral compounds. Therefore, these analytes are
42 commonly converted into various derivatives prior to separation of their enantiomers on
43 polysaccharide-based chiral columns [4-10]. Although derivatization of analytes provides
44 certain advantages from the viewpoint of separation (improved peak shape, additional
45 intermolecular interactions) and detection, it also has disadvantages such as an additional
46 synthetic step prior to separation, possibility of incomplete derivatization, interference of
47 residual derivatizing reagent in the separation process, additional costs, etc. In addition, if
48 the analyte is required in its intact but enantiomerically pure form after the separation,
49 recovering the analyte from its derivative used in the separation process in its initial form
50 will also require an additional step which may be burdened by additional shortcomings.
51 Since many amino acids do not possess strong UV-chromophores in their structures, in
52 earlier studies their derivatization was performed both, for improving their
53 enantioselectivity and also for improving their detectability with UV/VIS or
54 fluorescence detectors. However, with the successful detection of native amino acids by
55 alternative techniques such as mass spectrometry (MS), refractive index (RI), evaporative
56 light scattering (ELS), corona charged aerosol (CA) detectors or based on label-free
57 fluorescent detection, the derivatization of amino acids with the sole purpose of

58 improving their detectability becomes unnecessary. Thus, in some cases the separation of
59 native amino acids may offer certain advantages over the separation of their derivatives.

60 Several efficient chiral selectors are available for the separation of enantiomers of
61 native amino acids. Actually, the history of enantioselective high-performance liquid
62 chromatography (HPLC) started by the successful separation of enantiomers of native
63 amino acids reported by Davankov and Rogozhin in 1971 [11]. Today, the most useful
64 chiral selectors for the separation of enantiomers are cation- and anion-exchangers, as
65 well as zwitterionic chiral selectors developed by Lindner and co-workers [12-14],
66 macrocyclic antibiotic- and cyclofructane-based materials proposed by Armstrong and
67 co-workers [15-17], crown ether-based [18] and few other chiral stationary phases [19,
68 20]. In early 2000s Ye and co-authors have published the article reporting a separation of
69 underivatized amino acid enantiomers on polysaccharide-based chiral column, Chiralpak
70 AD in HPLC [21]. Later, Stringham reported separation of enantiomers of underivatized
71 proline and 4 aromatic amino acids, baclofen, 2-phenylglycine, phenylalanine and
72 tyrosine on the same chiral column in supercritical fluid chromatography (SFC) [22].
73 Separation of enantiomers of underivatized amino acids under SFC conditions on few
74 polysaccharide-based chiral columns was also reported in the conference presentation by
75 Yan and Riley [23].

76 In this manuscript our results are reported on the evaluation of several coated and
77 covalently immobilized polysaccharide-based chiral selectors for the separation of
78 enantiomers of 14 native amino acids, namely valine, norvaline, threonine, phenylalanine,
79 leucine, isoleucine, norleucine, lysine, alanine, histidine, serine, carnitine, oxoproline

80 (pyroglutamic acid) and aspartic acid (Fig. 1a) in sub/supercritical fluid chromatography
81 (SFC).

82

83 **2. Experimental part**

84 2.1 Materials

85 All studied amino acids, trimethylamine and n-butylamine were purchased from
86 Sigma-Aldrich (Steinheim, Germany) and Acros Organics (Illkirch, France) respectively.
87 HPLC grade methanol (MeOH) and ethanol (EtOH) were purchased from Carlo-Erba
88 Reagents (Val-de-Reuil, France). Carbon dioxide (CO₂) with purity of 99.995% was
89 purchased from Linde (Saint Priest, France). Chiral columns, such as Chiralpak AS based
90 on amylose tris((*S*)- α -phenylethylcarbamate) as chiral selector, Chiralcel OD-H and
91 Chiralpak IB based on cellulose tris(3,5-dimethylphenylcarbamate) as chiral selector,
92 Chiralpak AD-H and Chiralpak IA based on amylose tris(3,5-dimethylphenylcarbamate)
93 as chiral selector were purchased from Chiral Technologies Europe (Illkirch, France).
94 Lux Cellulose-2 based on cellulose tris(3-chloro-4-methylphenylcarbamate) as chiral
95 selector, Lux Amylose-2 based on amylose tris(5-chloro-2-methylphenylcarbamate) as
96 chiral selector and Lux i-Cellulose-5 based on cellulose (3,5-dichlorophenylcarbamate) as
97 chiral selector were provided by Phenomenex (Le Pecq, France). The structures of all
98 chiral selectors part of the studied columns are shown in Fig. 1b. All columns were 4.6 x
99 250 mm in size and packed with 5 micrometer particles.

100

101 2.2 Sample solutions

102 Screening solutions of racemic amino acids were prepared at 3 mg/mL
103 concentration in methanol/H₂O 50/50 (v/v) mixture, except for aspartic acid which was
104 prepared in 1 mg/mL concentration. The solutions were degassed by an ultrasonic bath
105 and filtered through a 0.45 µm PTFE syringe-filter (15 mm diameter) prior to be used.

106

107 2.3 Supercritical fluid chromatography apparatus

108 The SFC system used was an SFC-PICLAB hybrid 10-20 apparatus (PIC
109 Solution, Avignon, France). The pump head used for pumping the CO₂ was cooled to –
110 8° C by a cryostat (model Minichiller, Huber, Offenburg, Germany). The injection valve
111 was provided with a 19.6 µL sample loop. The system also included a Smartline 2600
112 diode array detector (DAD) (Knauer, Berlin, Germany). Detection wavelength was set at
113 210 nm. A Sedex 85 evaporative light scattering detector (ELSD) (Sedere, Alfortville,
114 France), was connected in-line with the DAD and right before the backpressure regulator
115 using a 0.064 mm ID × 160 cm long peek tubing. The pressure of the nebulizer gas (N₂)
116 was set at 3.7 bar. After optimization, the drift tube temperature was equal to 60° C and
117 the gain was set at 8. The system was controlled, and the data acquired and processed
118 with the SFC PicLab Analytic Online v.3.1.2 and Offline v.3.2.0 software (PIC Solution),
119 respectively. During the separation optimization, the mobile phase composition was
120 varied between 20- 40 % methanol or ethanol as modifiers in CO₂. The used flow rate
121 was 3 mL/min if not indicated otherwise. All analyses were run in isocratic mode. The
122 column oven temperature was 40°C and the outlet pressure was maintained at 15 MPa for
123 all experiments. The dead time was marked as the first disturbance of the baseline after

124 injection, caused by the solvent peak. Retention times were average values of two
125 replicate measurements.

126

127 **3. Results and Discussion**

128 3.1 Screening of polysaccharide-based chiral columns for the separation of
129 enantiomers of underivatized amino acids

130 SFC is well established technique for separation of enantiomers [24-27]. Due to
131 lower viscosity of and fast analyte diffusion in supercritical fluids this technique offers
132 the possibility of operating at higher flow rates (fast separations) as well as generating
133 higher plate numbers compared to HPLC. In addition, in preparative scale separations
134 removal of the mobile phase is easier and less costly compared to other separation modes.
135 This technique has been explored for the separation of derivatized amino acids also with
136 polysaccharide-based chiral columns [9, 10]. However, to the best of our knowledge only
137 one journal publication describes the separation of 5 underivatized amino acids, among
138 which 4 are aromatic, with polysaccharide-based chiral columns in SFC [22].

139 All analytes were injected on the eight columns with either 40% methanol or
140 ethanol as co-solvent. The separation was performed at 40°C. The results on
141 enantioseparation of the 14 amino acids on 8 polysaccharide-based chiral columns with
142 coated or covalently immobilized chiral selectors showed that some of the studied
143 columns did not provide separation of any pair of enantiomers under this study. In
144 particular, coated-type columns such as Chiralcel OD, Chiralpak AD and Chiralpak AS
145 belong to this category. In addition, no separation of enantiomers was observed on the
146 covalently immobilized version of Chiralcel OD and Chiralpak AD columns, Chiralpak

147 IB and Chiralpak IA, respectively. Lux Cellulose-2, having a chlorinated chiral selector
148 provided baseline separation for the enantiomers of phenylalanine, valine, isoleucine and
149 partial resolution of enantiomers of leucine (Data not shown). Lux Amylose-2 provided
150 partial resolution of enantiomers of phenylalanine (Data not shown). The best enantiomer
151 resolving ability among studied chiral columns was provided with Lux i-Cellulose-5
152 based on cellulose-(3,5-dichlorophenylcarbamate) as chiral selector in immobilized form
153 (Table 1). Some typical examples are shown in Fig. 2. The high enantiomer resolving
154 ability of cellulose tris(3,5-dichlorophenylcarbamate) has been known for many years
155 [28-32] and has been observed also for rather hydrophilic non-aromatic compounds, such
156 as chiral metal complexes [31, 32]. Considering this experience, the results observed in
157 the present study for non-aromatic amino acids do not seem surprising.

158

159 **3.2 Effect of mobile phase optimization**

160 3.2.1 Type of co-solvent

161 The mobile phase was CO₂/modifier mixtures; firstly 40% of either methanol or
162 ethanol were tested. As it can be seen from Fig. S1, for the enantiomers of norvaline and
163 leucine, ethanol was preferable modifier. Thus, ethanol has been used in further studies.

164

165 3.2.2 Use of mobile phase additive

166 In liquid-phase separations of enantiomers with polysaccharide-based chiral columns
167 commonly basic additives are used for the separations of basic compounds and acidic
168 additives for acids. The mixture of a basic and an acidic additive has also been used [33-
169 35]. In SFC, due to the acidic nature of carbon dioxide [36] especially in the presence of

170 alcohols [37], for the acidic solutes the addition of an acidic additive in the mobile phase
171 is not as common as in HPLC. Triethylamine (TEA) in the amount 0.3% (v/v) was used
172 as the mobile phase additive in initial experiments. As shown in Fig. S2a the additive
173 enabled good baseline separation of enantiomers of norvaline, but broad peaks were
174 observed. Similar results were obtained for enantiomers of valine. For enantiomers of
175 leucine only partial separation was observed. Further adjustment of the ethanol content
176 and increasing the concentration of TEA in the mobile phase following the recently
177 published work by Speybrouck *et al.* [38] enabled to improve peak shapes and resolution
178 further (Figs. S2b-f). Thus, except for phenylalanine and histidine, for which 0.3% of
179 TEA additive was used, 3% TEA allowed a good compromise between peak shape,
180 analysis time and peak resolution and was therefore chosen.

181 In order of further improving peak shapes, EtOH-water mixtures in different ratios
182 was applied as mobile phase component. The use of water in the mobile phase can
183 suppress peak tailing by counteracting the limited solubility of amino acids in CO₂-based
184 mobile phases. The use of 2 or 5% water in MeOH with chiral polysaccharide-based
185 stationary phases has been reported earlier [39]. Thus, a mobile phase of 20% (2% H₂O
186 and 3% TEA in EtOH)/CO₂ was tested but did not improve resolution significantly.

187 Lastly, whenever analysis times were too long, the flow-rate was increased from 3
188 mL/min to 4 mL/min for isoleucine, phenylalanine and carnitine and to 6 mL/min in case
189 of histidine. The best results obtained for the ten amino-acids are summarized in Table 1.

190

191 3.3 Evaluation of the potential contribution of ELSD to extra-column band-
192 broadening

193 ELSD is a useful universal detector for non-aromatic amino acids having weak
194 UV-absorbance. However, this detector may generate additional extra-column band-
195 broadening. In order to study this phenomenon, the separation of phenylalanine
196 enantiomers on Lux i-Cellulose-5 was monitored with sequentially connected UV-VIS
197 and ELS detectors in a rather wide flow-rate range (from 2 to 8 mL/min). Selected
198 chromatograms are shown in Fig. 3 for medium and high flow-rates. As it can be seen
199 from these overlaid chromatograms, ELSD does not contribute significantly to extra-
200 column band-broadening, at least not in this particular case.

201

202 **4. Conclusions**

203 As this study illustrates polysaccharide-based chiral columns may successfully be
204 used for the separation of enantiomers of underivatized nonaromatic amino acids in SFC.
205 As expected, the nature of the chiral selector and the composition of the mobile-phase
206 play key roles for differentiation of enantiomers. Among studied chiral columns Lux i-
207 Cellulose-5 having cellulose-(3,5-dichlorophenylcarbamate) as the chiral selector
208 exhibited most wide enantiomer-resolving ability towards studied amino acids. ELSD did
209 not cause any significant extra-column band-broadening.

210

211 ***Acknowledgement***

212 Anca-Elena Dascalu is grateful to the Fondation de la Catho for her PhD-scholarship. Dr.
213 Alina Ghinet is recognized for her gift of some amino acids used in this study.

214

215 **References**

- 216 [1] J. Shen, Y. Okamoto, Efficient separation of enantiomers using stereoregular
217 chiral polymers, *Chem. Rev.* 116 (2016) 1094-1138.
- 218 [2] B. Chankvetadze, Recent developments on polysaccharide-based chiral stationary
219 phases for liquid-phase separation of enantiomers. *J. Chromatogr. A* 1269 (2012)
220 26-51.
- 221 [3] H. Leek, L. Thunberg, A.C. Jonson, K. Öhlén, M. Klarqvist, Strategy for large-
222 scale isolation of enantiomers in drug discovery, *Drug Discovery Today* 22 (2017)
223 133-139.
- 224 [4] L. Chankvetadze, N. Ghibradze, M. Karchkhadze, L. Peng, T. Farkas, B.
225 Chankvetadze, Enantiomer elution order reversal of Fmoc-isoleucine by
226 variation of mobile phase temperature and composition, *J. Chromatogr. A* 1218
227 (2011) 6554-6560.
- 228 [5] P. López-Ram-de-Víu, J.A. Gálvez, M.D. Díaz-de-Villegas, High-performance
229 liquid chromatographic enantioseparation of unusual amino acid derivatives with
230 axial chirality on polysaccharide-based chiral stationary phases, *J. Chromatogr. A*.
231 1390 (2015) 78-85.
- 232 [6] J.Y. Jin, S.K. Bae, W. Lee, Comparative studies between covalently immobilized
233 and coated chiral stationary phases based on polysaccharide derivatives for
234 enantiomer separation of N-protected alpha-amino acids and their ester
235 derivatives, *Chirality* 21 (2009) 871-877.
- 236 [7] J.Y. Jin, K.A. Lee, J.S. Kang, Y.K. Kang, C.S. Baek, W. Lee, Comparison of
237 several polysaccharide-derived chiral stationary phases for the enantiomer

- 238 separation of N-fluorenylmethoxycarbonyl alpha-amino acids by HPLC, Arch.
239 Pharm. Res. 30 (2007) 659-664.
- 240 [8] K.A. Lee, S. Yeo, K.H. Kim, W. Lee, J.S. Kang, Enantioseparation of N-
241 fluorenylmethoxycarbonyl alpha-amino acids on polysaccharide-derived chiral
242 stationary phases by reverse mode liquid chromatography, J. Pharm. Biomed.
243 Anal. 46 (2008) 914-919.
- 244 [9] S. Khater, B. Canault, T. Azzimani, P. Bonnet, C. West, Thermodynamic
245 enantioseparation behavior of phenylthiohydantoin-amino acid derivatives in
246 supercritical fluid chromatography on polysaccharide chiral stationary phases, J
247 Sep. Sci. 41 (2018) 1450-1459.
- 248 [10] C. West, A. Bouet, I. Gillaizeau, G. Coudert, M. Lafosse, E. Lesellier, Chiral
249 separation of phosphine-containing α -amino acid derivatives using two
250 complementary cellulosic stationary phases in supercritical fluid chromatography,
251 Chirality 22 (2010) 242-251.
- 252 [11] S.V. Rogozhin, V.A. Davankov, Ligand chromatography on asymmetric complex-
253 forming sorbents as a new method for resolution of racemates, J. Chem. Soc. D:
254 Chem. Commun. 10 (1971) 490.
- 255 [12] I. Ilisz, A. Bajtai, W. Lindner, A. Péter, Liquid chromatographic enantiomer
256 separations applying chiral ion-exchangers based on Cinchona alkaloids, J.
257 Pharm. Biomed. Anal. 159 (2018) 127-152.

258

259

- 260 [13] F. Ianni, R. Sardella, A. Carotti, B. Natalini, W. Lindner, M. Lämmerhofer,
261 Quinine-based zwitterionic chiral stationary phase as a complementary tool for
262 peptide analysis: Mobile phase effects on enantio- and stereoselectivity of
263 underivatized oligopeptides, *Chirality* 28 (2016) 5-16.
- 264 [14] C.V. Hoffmann, R. Pell, M. Lämmerhofer, W. Lindner, Synergistic effects on
265 enantioselectivity of zwitterionic chiral stationary phases for separations of chiral
266 acids, bases, and amino acids by HPLC, *Anal. Chem.* 80 (2008) 8780-8789.
- 267 [15] T. Orosz, N. Grecsó, G. Lajkó, Z. Szakonyi, F. Fülöp, D.W. Armstrong, I. Ilisz,
268 A. Péter, Liquid chromatographic enantioseparation of carbocyclic β -amino acids
269 possessing limonene skeleton on macrocyclic glycopeptide-based chiral stationary
270 phases, *J. Pharm. Biomed. Anal.* 145 (2017) 119-126.
- 271 [16] N.L.T. Padivitage, D.W. Armstrong, Sulfonated cyclofructan 6 based stationary
272 phase for hydrophilic interaction chromatography, *J. Sep. Sci.* 34 (2011) 1636-
273 1647.
- 274 [17] D.W. Armstrong, B. Zhang, Chiral stationary phases for HPLC, *Anal. Chem.* 73
275 (2001) 557A-561A.
- 276 [18] M.H. Hyun, Liquid chromatographic enantioseparations on crown ether-based
277 chiral stationary phases, 1467 (2016) 19-32.
- 278 [19] Chirex Application Note from Phenomenex:
279 [http://az621941.vo.msecnd.net/documents/410e95e5-8426-4785-b851-](http://az621941.vo.msecnd.net/documents/410e95e5-8426-4785-b851-045289628c05.pdf)
280 [045289628c05.pdf](http://az621941.vo.msecnd.net/documents/410e95e5-8426-4785-b851-045289628c05.pdf)

- 281 [20] M. Sajewicz, M. Gontarska, T. Kowalska, HPLC–DAD evidence of the
282 oscillatory chiral conversion of phenylglycine, *J. Chromatogr. Sci.* 52 (2014) 329–
283 333
- 284 [21] Y.K. Ye, B. S. Lord, L. Yin, R. W. Stringham, Enantioseparation of amino acids
285 on a polysaccharide-based chiral stationary phase, *J. Chromatogr. A* 945 (2002)
286 147-159.
- 287 [22] R.W. Stringham, Chiral separation of amines in subcritical fluid chromatography
288 using polysaccharide stationary phases and acidic additives, *J. Chromatogr. A*,
289 1070 (2005) 163-170.
- 290 [23] T.Q. Yan, F. Riley, Presentation at PittCon 2017, March 5-9, 2017, Chicago, IL,
291 USA.
- 292 [24] C. West, Enantioselective separations with supercritical fluids, *Curr. Anal. Chem.*
293 10 (2014) 99-120.
- 294 [25] D. Speybrouck, E. Lipka, Preparative supercritical fluid chromatography: A
295 powerful tool for chiral separations, *J. Chromatogr. A* 1467 (2016) 33-55.
- 296 [26] K. De Klerck, Y. Vander Heyden, D. Mangelings, Exploratory data analysis as a
297 tool for similarity assessment and clustering of chiral polysaccharide-based
298 systems used to separate pharmaceuticals in supercritical fluid chromatography, *J*
299 *Chromatogr. A* 1326 (2014) 110-124.
- 300 [27] C. West, M.-L. Konjaria, N. Shashviashvili, E. Lemasson, P. Bonnet, R. Kakava,
301 A. Volonterio, B. Chankvetadze, Enantioseparation of novel chiral sulfoxides on
302 chlorinated polysaccharide stationary phases in supercritical fluid
303 chromatography, *J. Chromatogr. A* 1499 (2017) 174-182.

- 304 [28] Y. Okamoto, M. Kawashima, K. Hatada, Chromatographic resolution: XI.
305 Controlled chiral recognition of cellulose triphenylcarbamate derivatives
306 supported on silica gel, *J. Chromatogr.* 363 (1986) 173-186.
- 307 [29] B. Chankvetadze, C. Yamamoto, Y. Okamoto, Extremely high enantiomer
308 recognition in HPLC separation of racemic 2-(benzylsulfinyl)benzamide using
309 cellulose tris(3,5-dichlorophenylcarbamate) as a chiral stationary phase, *Chem.*
310 *Lett.* 29 (2000) 1176-1177.
- 311 [30] B. Chankvetadze, C. Yamamoto, Y. Okamoto, HPLC Enantioseparation with
312 cellulose tris(3,5-dichlorophenylcarbamate) in aqueous methanol as a mobile
313 phase, *Chem. Lett.* 29 (2000) 352-353.
- 314 [31] B. Chankvetadze, C. Yamamoto, Y. Okamoto, Enantioseparations using cellulose
315 tris(3,5-dichlorophenylcarbamate) in high-performance liquid chromatography in
316 common size and capillary columns: potential for screening of chiral compounds,
317 *Comb. Chem. High Throughput Screen.* 3(2000) 497-508.
- 318 [32] B. Gallinella, L. Bucciarelli, L. Zanitti, R. Ferretti, R. Cirilli, Direct separation of
319 the enantiomers of oxaliplatin on a cellulose-based chiral stationary phase in
320 hydrophilic interaction liquid chromatography mode, *J. Chromatogr. A* 1339
321 (2014) 210-213.
- 322 [33] K. De Klerck, G. Parewyck, D. Mangelings, Y. Vander Heyden, Enantioselectivity
323 of polysaccharide-based chiral stationary phases in supercritical fluid
324 chromatography using methanol-containing carbon dioxide mobile phases, *J.*
325 *Chromatogr. A* 1269 (2012) 336-345.

- 326 [34] K. De Klerck, C. Tistaert, D. Mangelings, Y. Vander Heyden, updating a
327 generic approach in sub- or supercritical fluid chromatography for
328 the enantioseparation of pharmaceuticals, *J. Supercrit. Fluids* 80 (2013) 50-59.
- 329 [35] L. Mosiashvili, L. Chankvetadze, T. Farkas, B. Chankvetadze, On the effect of
330 basic and acidic additives on the separation of the enantiomers of some basic
331 drugs with polysaccharide-based chiral selectors and polar organic phases, *J.*
332 *Chromatogr. A* 131 (2013) 167-174.
- 333 [36] C. West, J. Melin, H. Ansouri, M. Mengue Metogo, Unravelling the effects of
334 mobile phase additive in supercritical fluid chromatography. Part I: polarity and
335 acidity of the mobile phase, *J. Chromatogr. A* 1492 (2017) 136-143.
- 336 [37] A. Grand-Guillaume Perrenoud, J. Boccard, J-L. Veuthey, D. Guillarme, Analysis
337 of basic compounds by supercritical fluid chromatography: attempts to improve
338 peak shape and maintain mass spectrometry compatibility, *J. Chromatogr. A* 1262
339 (2012) 205-213.
- 340 [38] D. Speybrouck, C. Doublet, P. Cardinael, C. Fiol-Petit, D. Corens, The effect of
341 high concentration additive on chiral separations in supercritical fluid
342 chromatography, *J. Chromatogr. A* 1510 (2017) 89-99.
- 343 [39] J. Liu, E. L. Regalado, I. Mergelsberg, C. J. Welch, Extending the range of
344 supercritical fluid chromatography by use of water-rich modifiers, *Org. &*
345 *Biomolec. Chem.* 11 (2013) 4925-4929.

346

347 **Legends to the figures:**

348 **Fig. 1** Structure of (a) studied amino acids and (b) chiral selectors part of studied chiral
349 columns.

350 **Fig. 2** Separation of enantiomers of (a) valine, (b) norvaline, (c) isoleucine and (d)
351 norleucine on Lux i-Cellulose-5. Separation conditions were as shown in Table 1.

352 **Fig. 3** Chromatograms of phenylalanine enantiomers with the flow rates of the mobile
353 phase (a) 8 mL/min and (b) 4 mL/min when detected with DAD and ELSD. Other
354 separation conditions were as shown in Table 1.

355

356 **Supplementary Figures:**

357 **Figure S1** Chromatograms of Leucine and Norvaline with either MeOH or EtOH as
358 co-solvent in 60% CO₂, 3 mL/min, 40°C; 15 MPa back pressure; T_{ELSD} 60°C; gain
359 = 8.

360 **Figure S2** Separation of enantiomers of norvaline on Lux i-Cellulose-5, with 20%
361 EtOH and a) 0.3 %; b) 1.0% ; c) 2.0%; d) 3.0% ; e) 5.0% and f) 10% of TEA
362 additive, at 4 mL/min, 40°C, 15 MPa, T_{ELSD} 60°C, gain 8.

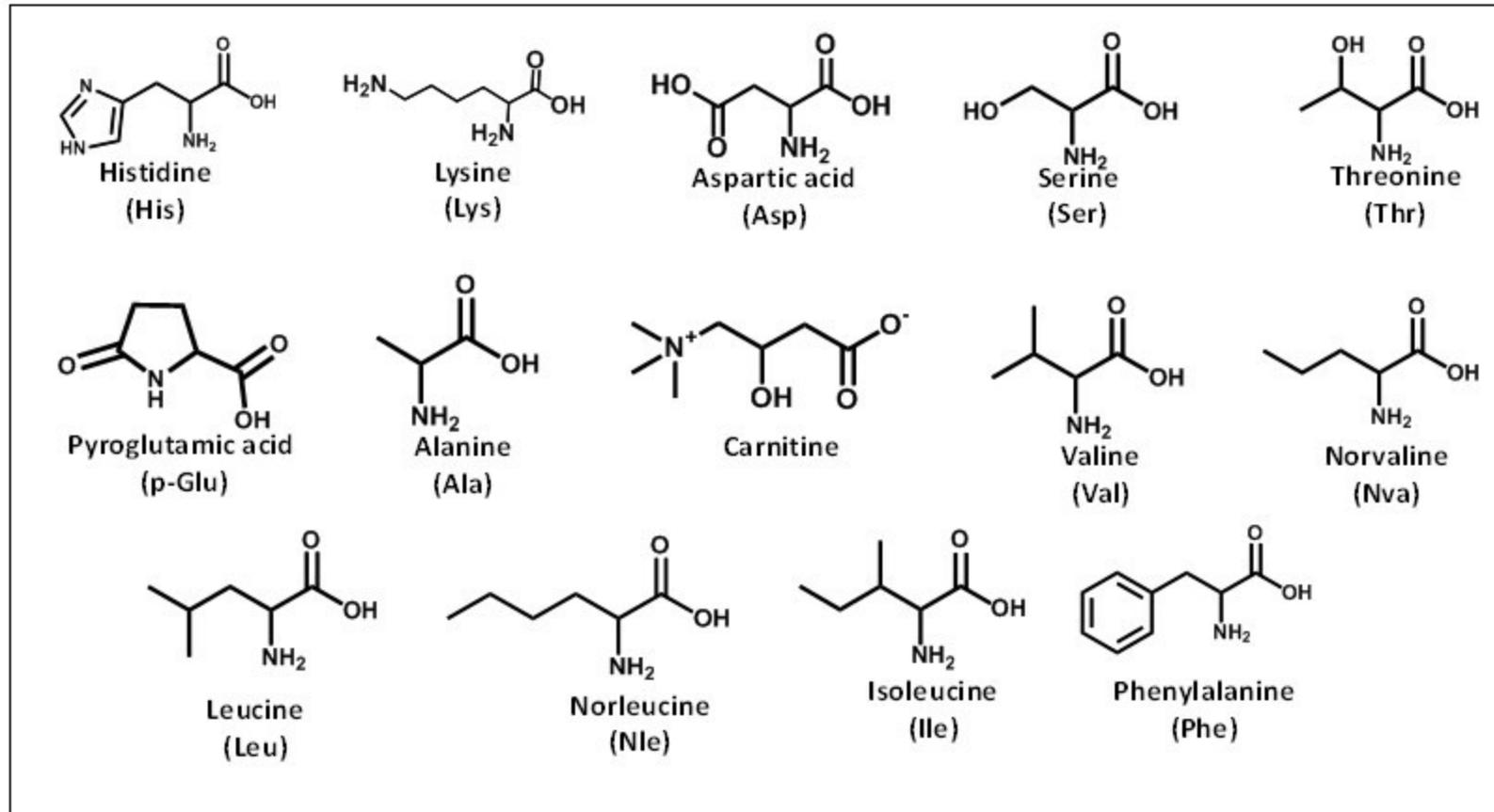
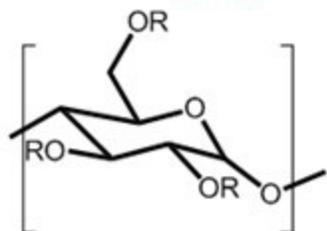
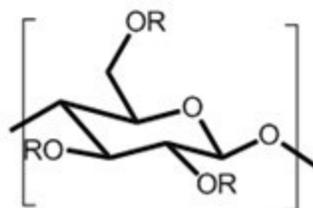


Fig. 1a

Amylose-based



Cellulose-based



Name	Nature	Selector
Chiralpak® AD	Coated	
Chiralpak® AS	Coated	
Lux™ Amylose-2	Coated	
Chiralpak® IA	Immobilized	

Name	Nature	Selector
Chiralcel® OD	Coated	
Lux™ i Cellulose-5	Immobilized	
Lux™ Cellulose-2	Coated	
Chiralpak® IB	Immobilized	

Fig. 1b

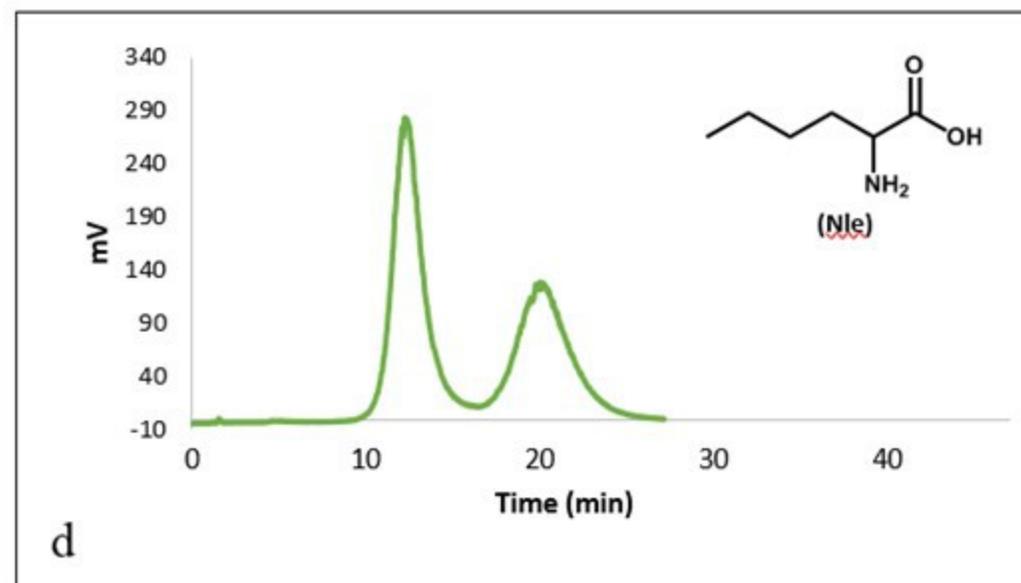
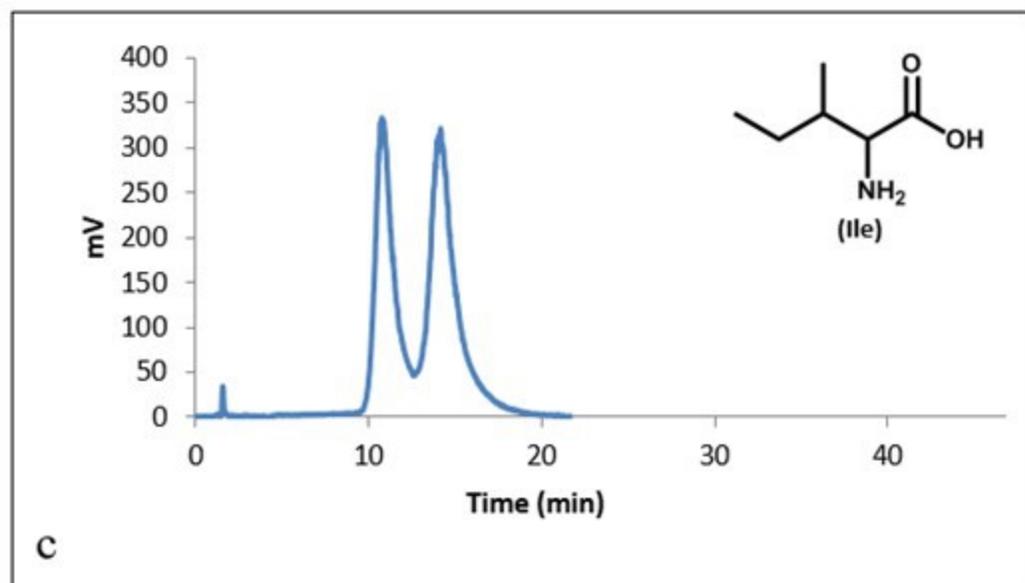
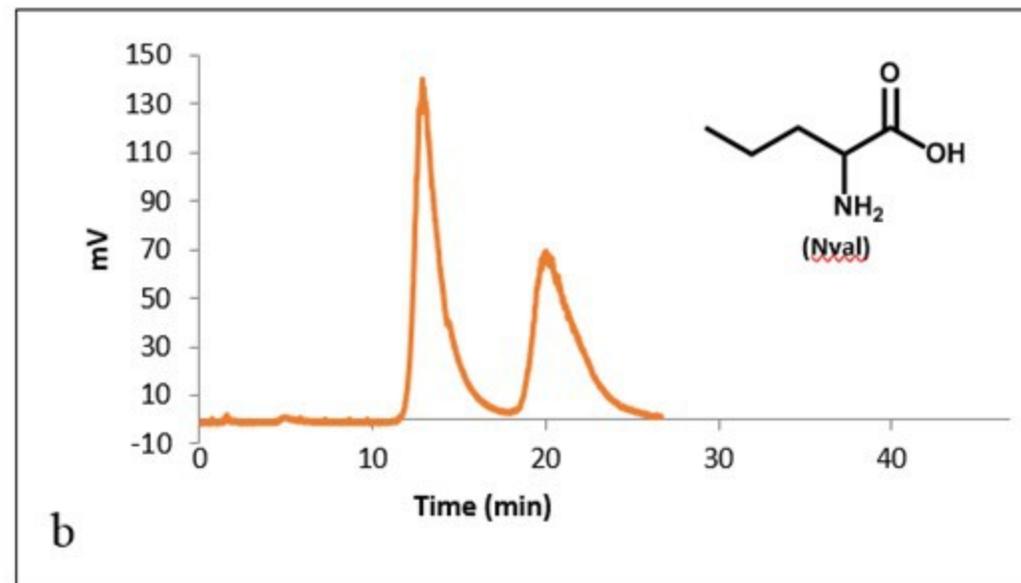
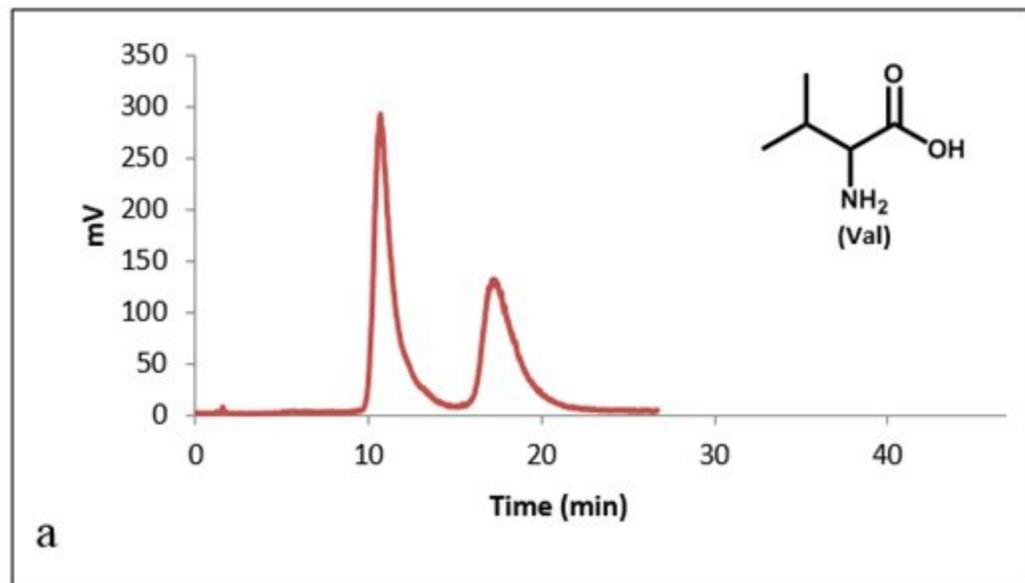


Fig. 2

Table 1: Chromatographic parameters of the separation of underivatized amino acids under optimized conditions by SFC with ELS detection on Lux i-Cellulose-5.

Amino acid	Conditions	tr ₁ (min)	tr ₂ (min)	k ₁	k ₂	α	Rs
Leucine	20% EtOH+TEA 3%, 3mL/min ^{a)}	11.44	16.05	6.15	9.00	1.49	1.63
Isoleucine	20% EtOH+TEA 3%, 4mL/min	10.51	15.43	5.57	8.64	1.55	1.61
Norleucine	20% EtOH+TEA 3%, 3mL/min	12.23	19.98	7.09	12.23	1.72	2.17
Phenylalanine	20% EtOH+TEA 0.3%, 4mL/min	14.62	20.46	11.72	16.79	1.43	1.59
Alanine	20% EtOH+TEA 3%, 3mL/min	16.08	23.76	10.08	15.38	1.50	1.53
Valine	20% EtOH+TEA 3%, 3mL/min	10.55	16.90	5.65	9.65	1.71	2.24
Norvaline	20% EtOH+TEA 3%, 3mL/min	12.90	19.59	6.77	10.80	1.60	1.83
Serine	20% EtOH+TEA 3%, 3mL/min	13.12	15.25	6.41	7.62	1.19	1.51
Carnitine	40% EtOH+TEA 3%, 4mL/min	11.12	16.21	10.34	15.54	1.50	1.95
Histidine	30% EtOH+TEA 0.3%, 6mL/min	14.37	21.47	16.96	26.17	1.25	1.99

a) Other experimental conditions were: The oven temperature 40°C, pressure 150 bar; T_{ELSD}= 60°C; gain = 8.