1 *Schinus terebinthifolius* **countercurrent chromatography (Part III): Method**
2 **transfer from small CCC column to preparative CPC ones as a part of method**
3 **development**

4

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14

15 **Abstract**

16 Countercurrent chromatography (CCC) and centrifugal partition chromatography (CPC)
17 are support free liquid-liquid chromatography techniques sharing the same basic
18 principles and features. Method transfer has previously been demonstrated for both
19 techniques but never from one to another. This study aimed to show such a feasibility
20 using fractionation of *Schinus terebinthifolius* berries dichloromethane extract as a case
21 study. Heptane – ethyl acetate – methanol –water (6:1:6:1, v/v/v/v) was used as solvent
22 system with masticadienonic and 3β-masticadienolic acids as target compounds. The
23 optimized separation methodology previously described in Part I and II, was scaled up
24 from an analytical hydrodynamic CCC column (17.4 mL) to preparative hydrostatic
25 CPC instruments (250 mL and 303 mL) as a part of method development. Flow-rate and

sample loading were further optimized on CPC. Mobile phase linear velocity is suggested as a transfer invariant parameter if the CPC column contains sufficient number of partition cells.

Key-words: countercurrent chromatography, centrifugal partition chromatography, method transfer, *Schinus terebinthifolius.*

1. Introduction

Since introduction of support-free liquid-liquid chromatography in 60-ties, the first apparatus based on gravitational force (droplet countercurrent chromatography – DCCC – and rotational locular countercurrent chromatography – RLCC) have been replaced by more efficient equipment which uses centrifugal force to hold the stationary liquid phase [1]. These modern and widely used techniques are countercurrent chromatography (CCC) and centrifugal partition chromatography (CPC). They use hydrodynamic and hydrostatic columns, respectively [2,3].

CCC (hydrodynamic support-free liquid-liquid chromatography) uses a variable centrifugal acceleration produced by a two-axis rotation, mimicking the planetary motion. The column is a Teflon or stainless steel tubing wrapped around a bobbin (holder), where centrifugal force changes in intensity and direction thus producing alternating mixing and settling zones [3-5]. On the other hand, CPC (hydrostatic support-free liquid-liquid chromatography) uses a constant centrifugal acceleration produced by a single-axis rotation. A CPC column is a series of partition cells connected by ducts (narrow channels) in cascades and arranged in a centrifuge. The stationary liquid phase is maintained inside the cells by the constant centrifugal acceleration while the mobile phase is pumped through it. Recently, new CPC devices,

called Centrifugal Partition Extractors (CPE) by the manufacturers, have been developed. The design of their column derives from classical CPC but with less cells of larger volume when compared to CPC with an equivalent column capacity, thereby facilitating mass overloading conditions and the use of high flow rate [6,7]. Thus, the CPE column are often presented as highly productive. [8].

Both support free liquid-liquid chromatography techniques present the same separation principles and features with some differences between them [9]: hydrostatic columns have excellent stationary phase retention inside the cells although restricted by the dead volume corresponding to the connecting ducts, even with biphasic solvent systems with low density difference and/or high viscosity. It is possible to work at high flow-rates but with significant back-pressure, depending of the number of partition cells and the physico-chemical properties of the solvents. Hydrodynamic columns also provide excellent stationary phase retention, especially for intermediate polarity systems and can easily cope with crude/viscous samples containing particles. CCC columns work at much lower pressure, though stationary phase retention with biphasic solvent systems with low density difference and/or high viscosity can be more difficult.

Choosing a correct solvent system is the most important step when working with CCC or CPC. A common approach is based on searching the literature for solvents systems that have been used for the purification of similar compounds [10]. Following this pathway, it is not rare to find a solvent system and method for CPC while working with CCC or *vice versa*. The aim of this paper is to define a methodology to transfer experimental conditions from a small CCC column (17.4 mL) to a CPC one with higher volume (CPC and CPE types).

This approach can be helpful for quick testing of experimental conditions on an analytical CCC device to reduce sample and solvent consumption, for transfer to semi-

76 preparative/preparative CPC/CPE instruments. Indeed, the smallest CPC column
77 available on the market has a column capacity of about 30 mL, while CCC column can
78 be as small as 5mL.
79

80 2. Experimental

81 *2.1 Materials*

82 All solvents – Heptane (Hep), ethyl acetate (EtOAc), methanol (MeOH), acetonitrile
83 (CH_3CN) – were purchased from Carlo Erba Reactifs SDS (Val de Reuil, France).
84 Deionized water was used to prepare aqueous solutions.
85 *Schinus terebinthifolius* berries dichloromethane extract, solvent system and sample
86 preparation methodology was taken from a previous work [11].
87

88 *2.2 CCC and CPC instruments*

89 Three support-free liquid-liquid instruments were used in this work:
90 Mini DE centrifuge (Dynamic Extractions, Tredegar, UK) equipped with a
91 polytetrafluorethylene (PTFE) multi-layer column (17.4 mL and 0.8 mm i.d.). The
92 distance between the central rotor axis and the column axis is 50 mm. The β-value
93 ranges from 0.50 to 0.76 and the rotation speed is adjustable from 200 to 2100 rpm
94 producing *g* field reaching 500g level at periphery of the column. The system is
95 equipped with an Agilent HP1100 (Santa Clara, California, U.S.A.) pump and a Foxy
96 Jr, Teledyne Isco (Lincoln, Nebraska, U.S.A.) fraction collector.
97 The FCPE300 device (Kromaton Technology, Angers, France) was equipped with a
98 rotor of 7 stacked partition disks engraved with a total of 231 twin partition cells. The
99 total volume of the column is 303 mL and the volume of interconnecting cell ducts is 73
100 mL. The rotation speed can be adjusted from 500 to 2000 rpm, producing a relative

101 centrifugal acceleration in the partition cell up to 437 *g*. Phases were pumped with a
102 KNAUER Preparative Pump 1800 V7115 (Berlin, Germany). The system was coupled
103 to a UVD 170S detector (Dionex, Sunnivale, CA, USA) equipped with a preparative
104 flow cell. The eluent was monitored at 254 nm. Samples were injected through a sample
105 loop with volume varied according to **Tables 1** and **2**. Fractions were collected by a
106 Pharmacia Superfrac collector (Uppsala, Sweden). Chromatographic data were acquired
107 by using the Chromeleon Software version 6.11 (Dionex).
108 The CPC ASCPC250 (Armen Instrument, Vannes, France) was equipped with a 250
109 mL rotor containing 21 stacked discs with a total of 1890 twin-cells was used. The total
110 active volume is 214 mL (about 0.1 mL per cell) and the volume of interconnecting cell
111 ducts is 30 mL. Rotation speed could be adjusted from 500 to 3000 rpm, thus producing
112 a centrifugal force field in the partition cells up to 700 *g*. Samples were injected through
113 a sample loop. The solvents were pumped through a semi-preparative 4-way binary
114 high-pressure gradient Armen Light version pump (50 mL/min maximum flow-rate, 150
115 bars). The detection was done by UV Armen Detector at 254 nm. Fractions were
116 collected by an Armen Fraction Collector LS-5600. Chromatographic data were
117 acquired by using the Armen Glider CPC Control Software V2.9.2.9
118
119 *2.3 CCC and CPC procedure*
120 All experiments were performed using upper organic phase as a stationary phase
121 (reversed phase in CCC and corresponding descending mode in CPE/CPC). The system
122 was first completely filled with the stationary phase. Rotation was set to 2100 rpm in
123 Mini-DE and 1200 rpm in FCPE300 and ASCPC250. For the separation, the lower
124 aqueous mobile phase was pumped at a flow-rate specified in **Table 2**. After reaching
125 hydrodynamic equilibrium, the sample dissolved in both solvent phases (1:1, v/v) was

126 injected to a column using an injection valve (for sample loading, see **Table 2**). For the
127 elution step, one column volume of mobile phase was pumped through and fractions
128 were collected in 1 min intervals. For the extrusion step in CCC, rotation was reduced to
129 200 rpm in Mini-DE. In case of FCPE300 and ASCPC250, rotation was maintained at
130 1200 rpm with mobile phase pumped in ascending mode for back-extrusion of column
131 content [12].

132

133 *2.4 Scale-up factor calculation from analytical CCC to preparative CPE/CPC*

134 A volumetric scale-up factor was applied to transfer parameters from CCC Mini-DE
135 [13] to CPE FCPE300 and CPC ASCPC250 equipment, as follows:

136 $$Volumetric\ SUF = \frac{V2}{V1} \quad \text{(Eq. 1)}$$

137

138 *2.5 Stationary phase retention, efficiency and mobile phase linear velocity calculation*

139 For the CPC columns, stationary phase retention (Sf) was expressed as follows:

140

141 $$Sf = V_S / V_{cell}\ ;\ \text{being}\ V_S = V_{cell} - V_M \quad \text{(Eq. 2)}$$

142

143 where V_S is the stationary phase volume, V_{cell} is the total partition cell volume in the
144 column (where the transfer phenomena take place) and V_M is the mobile phase volume.

145

146 $$V_{cell} = V_{column} - V_{ducts} \quad \text{(Eq. 3)}$$

147

148 Efficiency (N) was calculated using the following formula:

149

150 $$N = 16 \left(\frac{V}{W}\right)^2 \quad \text{(Eq. 4)}$$

151

152 Where V is the peak volume and W is the width volume of the compound.

153 For the CCC column, the linear velocity of the mobile phase (u_{CCC}) can be easily

154 calculated from the Sf value:

155

156 $$u_{CCC} = \frac{4F}{\pi d^2(1-Sf)} \quad \text{(Eq. 5)}$$

157

158 with F the flow-rate in $cm^3.min^{-1}$ and d the tubing internal diameter of the PTFE tubing

159 in cm to obtain mobile phase linear velocity in $cm.min^{-1}$.

160 For the CPC column, an average cross section ($\overline{CS}$) has to be calculated since the

161 column is made of a succession of partition cells with a particular design: the twin-cells:

162

163 $$\overline{CS} = \frac{v_{cell}}{h_{cell}} \quad \text{(Eq. 6)}$$

164

165 where v_{cell} and h_{cell} are the volume in cm^3 and the height in cm of a single partition cell

166 (data from manufacturers), respectively.

167 Then, the mobile phase linear velocity for a CPC column in $cm.min^{-1}$ (u_{CPC}) can be

168 calculated as:

169

170 $$u_{CPC} = \frac{F}{\overline{CS}(1-Sf)} \quad \text{(Eq. 7)}$$

171

172 **Table 1** presents the calculated mobile phase linear velocities for the Mini-DE CCC, the

173 ASCPC250 and the FCPE300 columns.

174

175 **Table 1.** Equipment details and experimental conditions

176

177 *Insert Table 1 here*

178

179 *2.6 Analyses of obtained CCC fractions*

180 CPE and CPC collected fractions were analyzed by TLC (Merck Art. 05554, Darmstadt,
181 Germany) developed with chloroform-ethyl acetate 3:1 (v/v). Plates were sprayed with
182 universal reagent (50% H_2SO_4 and 50% vanillin solution, both in water) followed by
183 heating. Results were compared to previous TLC analysis [11] to identify the target
184 compounds.

185 Aliquots of 200µL of selected fractions from CPE and CPC selected experiments were
186 dried under reduced pressure for further HPLC analyses. A Thermofisher Ultimate 3000
187 (Thermo Fischer Scientific, Villebon sur Yvette, France) was used, equipped with a 4
188 ways pump LPG 3400 SD, an automatic injector WPS 3000 SL and a UV/Visible
189 detector DAD 3000. The column was a BEH C18, 50 × 2.1 mm i.d., 1.7µm particle
190 size). The flow rate was 0.4 mL/min. The mobile phase was composed of TFA 0.025%
191 in water (solvent A) and CH_3CN (solvent B). The gradient was performed by increasing
192 solvent B from 75% to 90% in 15 min. UV detection was monitored at 210 nm. Data
193 were acquired with Chromeleon software, version 6.0.1 (Dionex, USA).

194

195 *2.7 Resolution calculation*

196 Resolution (Rs) was calculated using the following formula:

197 $$Rs = \frac{2\,(V2-V1)}{W2+W1} \qquad \text{(Eq. 8)}$$

198 Where V is the peak volume and W is the width volume of two consecutive compounds.
199 This calculation was based on TLC analysis or HPLC fractograms. In the case of the

200 use of TLC fractograms, the same volume of each CCC or CPC fraction was carefully
201 spotted on TLC plates (for detailed information, see [11, 13]).
202
203 **3. Results and discussion**
204 Scale up in support-free liquid-liquid chromatography is often described as a linear
205 process with the scale up factor being generally based on the volume ratio between
206 different CCC columns. Recently, a scale change study integrating hydrodynamics
207 aspects in CPC showed that it is not a linear phenomenon and that the hydrodynamic
208 aspects are very important [14].
209 The aim of present study was to transfer the purification methodology of two triterpene
210 acids, 3β-masticadienolic acid and masticadienonic acid (**Figure 1**) from *Schinus*
211 *terebinthifolius* [11, 13], from an analytical CCC column to preparative CPC columns.
212 This purification was achieved by using the biphasic solvent system heptane / ethyl
213 acetate / methanol / water 6:1:6:1 (v/v) with the lower aqueous phase as the mobile one.
214
215 *Insert here figure 1*
216
217 **Figure 1.** Chemical structures of the triterpene acids used as target compounds.
218
219 The transfer of the optimized purification from an analytical scale CCC – developed in
220 part I [11] – to a CPC instrument with a larger capacity was done using scale up factor
221 (SUF) based on the column volume ratio – introduced by the authors in Part II [13]
222 (**Table 2**). Two parameters, the flow rate and the sample loading were then increased.
223 The mobile phase linear velocity was calculated for each CCC and CPC operating
224 conditions to determine if this parameter is relevant for method transfer.

3.1 From MINI-DE CCC to ASCPC250 CPC

The methodology was transferred between a 17.4 mL hydrodynamic column with 200 loops (Mini-DE CCC) and a 250 mL (214 mL working volume) hydrostatic column equipped with 1890 partition cells (ASCPC250).

The first CPC experiment was carried out by using a SUF of 14 (250 divided by 17.4) for the flow rate and the sample loading (see **Table 2**). A flow rate of 7 mL.min-1 was thus used as starting point with the injection volume of 5 % of the column volume at a fixed sample concentration of 100 mg.mL^{-1}. To enhance the productivity, different flow rates were tested (7, 14, 21 and 28 mL.min^{-1}). The best combination of the run time and the target recovery corresponded to 21 mL.min^{-1} (**Figure S1** in Supplementary Material). Further increase of flow rate leads to drop in resolution with two target triterpenes partially co-eluting. In all cases, the flow rate for the extrusion step was double that used for the elution step to increase the productivity.

Table 2. Experimental details

Insert Table 2 here

As shown in **Table 1** (*Section 2.5*), the linear velocities of the mobile phase are similar for the purification on the Mini-DE and the ASCPC250 (398 cm.min^{-1} and 405 cm.min^{-1}, respectively). CCC and CPC are very close techniques which differ only in the column design and in this case linear velocity of the mobile phase appears to be a suitable transfer invariant.

249 The next step was optimization of the sample loading. The target compounds, both
250 triterpene acids, have emulsifying properties, which potentially can disturb
251 hydrodynamic equilibrium at high sample loading. **Figure 2** shows that a sample
252 loading up to 200 mg.mL^{-1} in 12 mL, 5 % of the column volume, (equivalent to 1.1 g /
253 100 mL Vc) can be reached without decrease in resolution, while at 400 mg.mL^{-1}
254 (equivalent to 2.2 g / 100 mL Vc) the two target triterpenes are partially co-eluting.
255 Only 100 mg.mL^{-1} (equivalent to 0.56 g / 100 mL Vc) sample concentration gives about
256 85% maximum purity for the first target. Any further increase leads to drop of
257 maximum purity below 70%, however, the maximum purity of the second target
258 remains in the region of 90%.
259 Optimisation of the sample volume at the fixed 200 mg.mL^{-1} concentration confirmed
260 that the chosen 5% of the column volume based on Part I [11] provides the best purity
261 of the target compounds (**Figure S2** in Supplementary Material).
262 *Insert here figure 2*
263
264 **Figure 2.** Fractogram of odd numbered fractions from CPC separations of *S.*
265 *terebinthifolius* berries (data shown only for target compounds). *Kd values of ionised
266 compounds might change if their pKa is close to the pH of a solvent system used [15].
267 See **Supplementary Material** for more information.
268
269 These results demonstrate CPC efficiency for the purification of samples having
270 surfactant properties due to column design. The selected biphasic solvent system
271 provides high selectivity for the separation of chosen target triterpenes with separation
272 factor of 2.25. This allows much higher loading with acceptable resolution and
273 therefore, - high productivity calculated per unit column volume. Nevertheless, the

274 efficiency calculated for 3β-masticadienolic acid is 60 (calculated from the HPLC
275 fractogram), that corresponds to approximately 32 twin-cells for one theoretical plate.
276 This low number of theoretical plates in the CPC column is probably due to the
277 emulsifying character of the two target compounds that limits their mass transfer
278 between the two liquid phases [16-18].

279

280 *3.2 From MINI-DE CCC to FCPE300 CPE*

281 For the method transfer from analytical CCC column to preparative FCPE300 extractor,
282 the same strategy was applied (first, optimization of the flow rate followed by
283 optimization of sample loading) with volumetric SUF of 17. After testing 9, 18, 27, 36
284 and 54 mL.min^{-}1, a flow rate of 27 mL.min^{-1} was selected, that corresponds to the best
285 combination of resolution and separation time (**Figure S3** in Supplementary Material).
286 Once again, a concentration of 200 mg.mL^{-1} in 15 mL, 5 % Vc (equivalent to 1 g / 100
287 mL column volume) can be loaded without significant decrease of the resolution
288 (**Figure 3**). The corresponding mobile phase linear velocity in the CPE column is only
289 174 cm.min^{-1}, which is 2.3 times less than in the CCC or CPC columns because the twin
290 cells of the CPE column are wider and thicker.

291

292 *Insert here figure 3*

293

294 **Figure 3.** TLC analyses of odd numbered fractions of the CPE separations of *S.*
295 *terebinthifolius* berries.

296

297 To utilize CPE to its full capacity it is better to use pH-zone refining or ion-exchange
298 elution modes, which are in general applicable for the separation of ionized compounds.

299 However, in this study, the separation between the two triterpene acids is achieved with
300 the isocratic reversed phase elution mode, therefore, the column efficiency is mainly
301 linked to the number of theoretical plates. The efficiency calculated on the basis of the
302 elution time at 27 $mL.min^{-1}$ and TLC analysis for 3β-masticadienolic acid and
303 masticadienonic acid were 23 and 34, respectively that leads to a resolution of 1.1.
304 Although, the overall efficiency of the 231 cell CPE column is not high enough for this
305 particular application in comparison to the CPC column (1890 cells), the individual
306 CPE cells (10 twin-cells per theoretical plate) are more efficient than the individual
307 CPC cells (32 twin-cells per theoretical plate). This supports the findings of Chollet *et*
308 *al.* [14]. The authors presented flow pattern visualizations which showed that larger
309 twin-cells led to more dispersed flow patterns at a given centrifugal acceleration. Thus,
310 in the CPE case, the mobile phase linear velocity cannot be used as transfer invariant
311 since the partition cell number limits dramatically the range of experimental conditions
312 in terms of flow rate. Therefore, the resulting productivity of 18 g of injected
313 sample/h/L of column is lower than the one obtained with the CPC column (37 g of
314 injected sample/h/L of column).
315

316 **4. Conclusions**

317 Method transfer between CCC and CPC (and *vice versa*) can meet different needs. The
318 aim is to adapt an experimental procedure found in the literature for the other technique
319 with or without scale change. On the other hand, it can be a question of method transfer
320 developed on a small CCC column to a CPC column with larger capacity. This work
321 clearly demonstrates that a simple transfer factor based on a volumetric approach is not
322 relevant since CCC and CPC columns have very different design. As an alternative,
323 mobile phase linear velocity can be used as the transfer invariant parameter assuming

324 that the CPC column contains enough partition cells for a chosen elution mode and the
325 average cross sectional area of a CPC cell equates with the cross-sectional area of a
326 CCC column. The sample loading should be optimized to improve productivity,
327 especially if the sample contains surfactants which reduces the interfacial tension and
328 therefore, might limit loading capacity of analytical CCC columns during method
329 development.

330

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338

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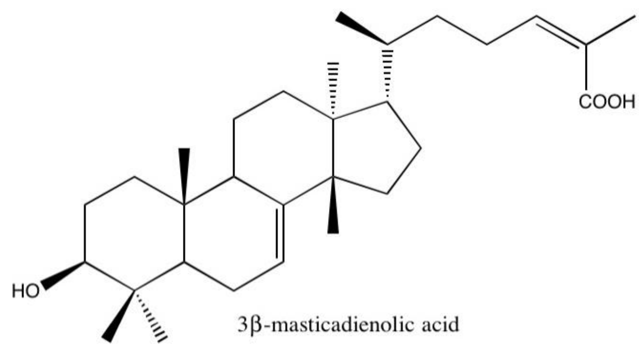

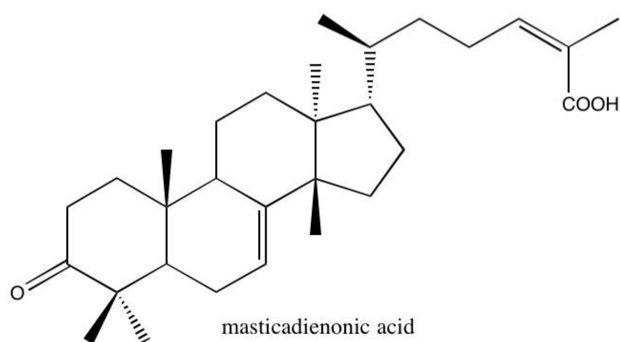

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396 **Figure 1.** Chemical structures of the triterpene acids used as target compounds.

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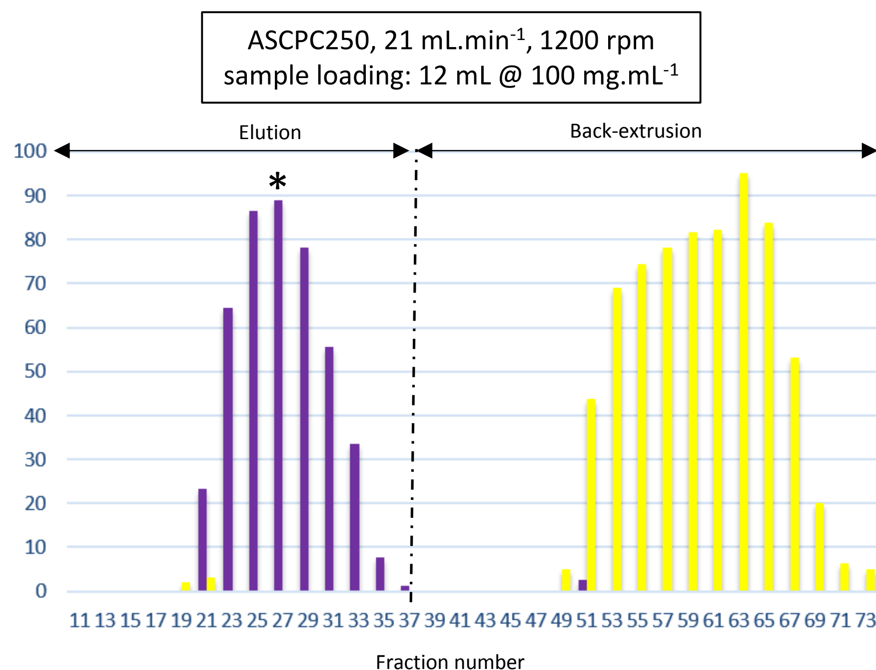

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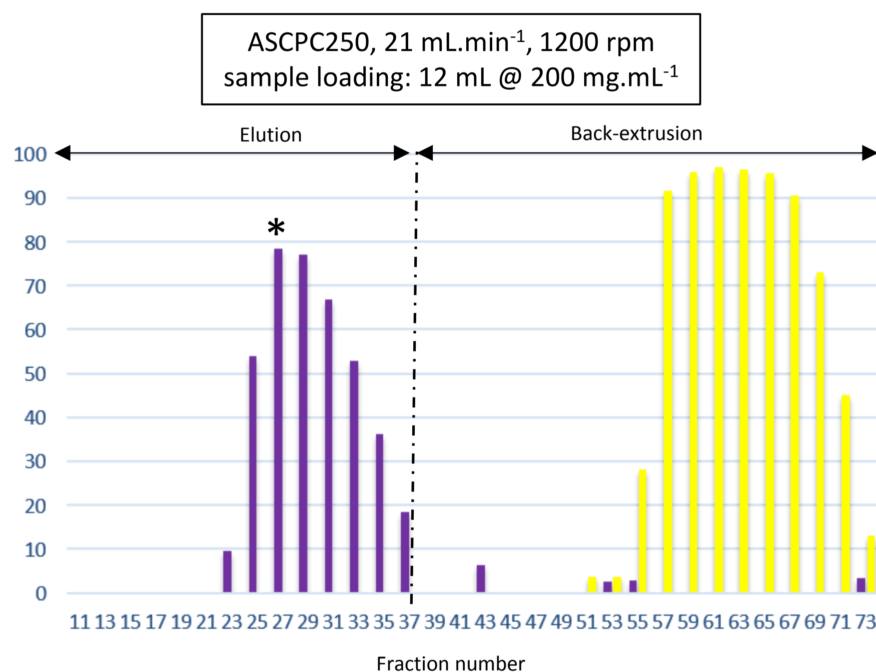

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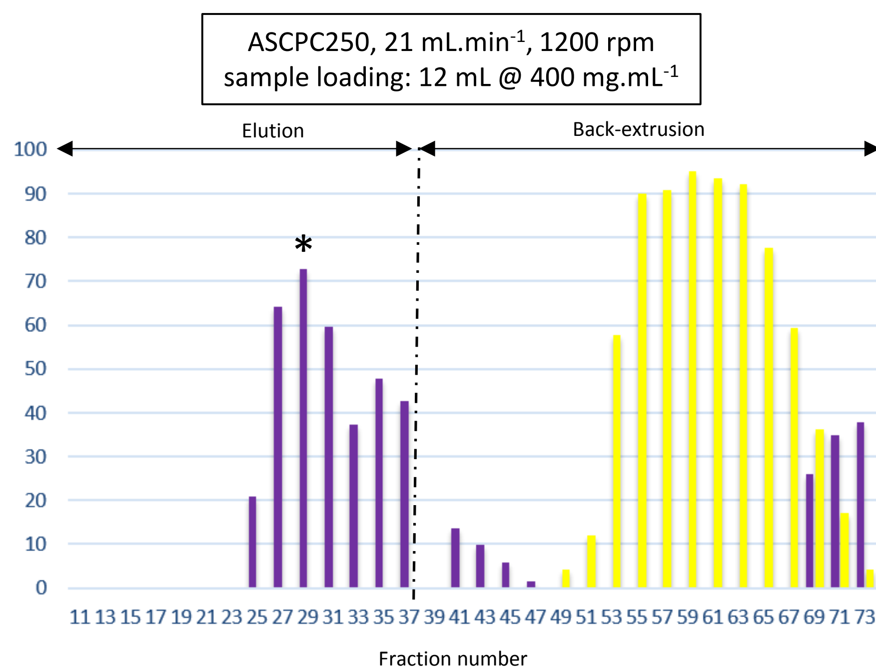
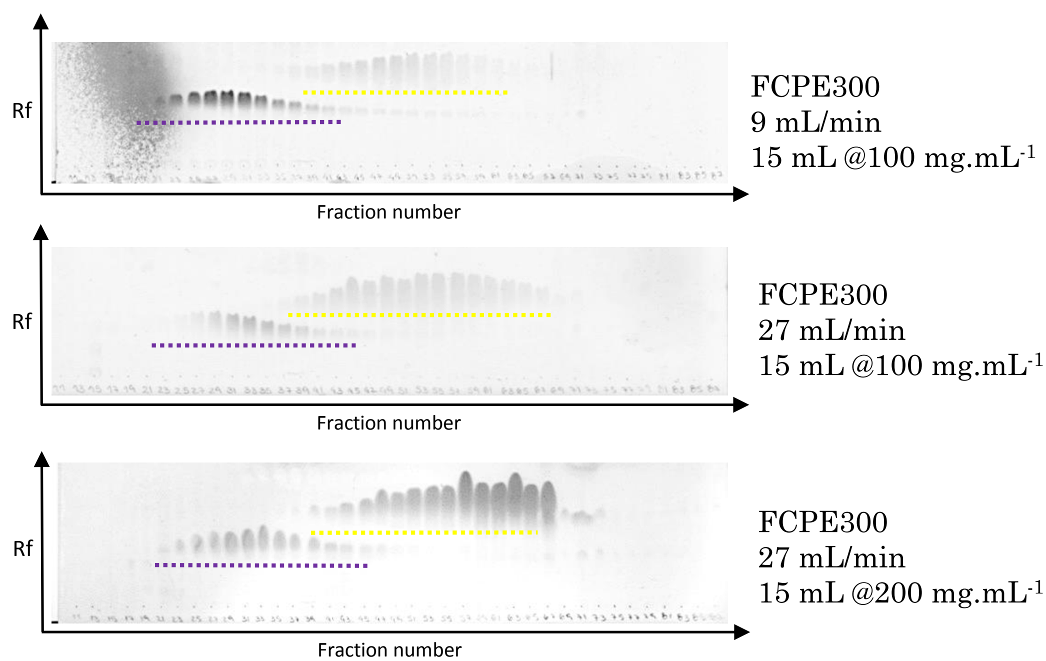


401 **Figure 2.** Fractogram of odd numbered fractions from CPC separations of *S.*
402 *terebinthifolius* berries (data shown only for target compounds). *Kd values of ionised
403 compounds might change if their pKa is close to the pH of a solvent system used [15].
404 See **Supplementary Material** for more information.
405



406

407 **Figure 3.** TLC analyses of odd numbered fractions of the CPE separations of *S.*
408 *terebinthifolius* berries.
409

Table 1. Equipment details and experimental conditions

	MiniDE CCC	ASCPC250	FCPE300
i.d. (cm)	0.08	-	-
Individual v_{cell} (cm^3)	-	0.10	0.90
h_{cell} (cm)	-	0.69	1.45
$\overline{CS}$ (cm^2)	-	0.14	0.62
Sf (in partition cells)	0.75	0.65	0.75
F ($cm^3.min^{-1}$)	0.5	21.00	27.00
u_{CCC} or u_{CPC} ($cm.min^{-1}$)	**397.88**	**405.00**	**174.00**
Injected sample / total partition cell volume ($mg.mL^{-1}$)*	4.88**	11.96	13.04

*The duct volume was remove from the calculation as it corresponds to a chromatographic dead volume.
**Injected sample/ column volume (no chromatographic dead volume in a CCC column)

Table 2. Experimental details

<table>
<tr><td>Instrument</td><td>Mini-DE CCC</td><td colspan="3">ASCPC250</td><td colspan="2">FCPE300</td></tr>
<tr><td>Column Volume (mL)</td><td>17.4</td><td colspan="3">250</td><td colspan="2">303</td></tr>
<tr><td>Solvent system</td><td colspan="6">Heptane/ethyl acetate/methanol/water (6 :1 :6 :1, v/v)</td></tr>
<tr><td>Elution mode</td><td>Reversed (extrusion after one Vc)</td><td colspan="5">Descending (back extrusion after one Vc)</td></tr>
<tr><td>Sample loading (1:1, SP/MP)</td><td>85 mg in 0.86 mL</td><td colspan="2">1.1 g in 12 mL</td><td>2.2 g in 12 mL</td><td colspan="2">3g in 15 mL</td></tr>
<tr><td>Flow rate of the elution –extrusion ($mL.min^{-1}$)</td><td>0.5 – 1</td><td>7 - 14</td><td colspan="2">21 – 42</td><td>9 – 9</td><td>27 - 27</td></tr>
<tr><td>Rotational speed (rpm)</td><td>2100</td><td colspan="5">1200</td></tr>
<tr><td>Sf (%)</td><td>75</td><td>86</td><td>67</td><td>65</td><td>88</td><td>75</td></tr>
<tr><td>Run duration (min)</td><td>50</td><td>54</td><td colspan="2">18</td><td>68</td><td>33</td></tr>
</table>

419 ***Schinus terebinthifolius* countercurrent chromatography (Part III): Method**
420 **transfer from small CCC column to preparative CPC ones as a part of method**
421 **development**

422

423 Fernanda das Neves Costa*, Jane Hubert, Nicolas Borie, Alexis Kotland, Peter
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426

427 **Supplementary data**

428

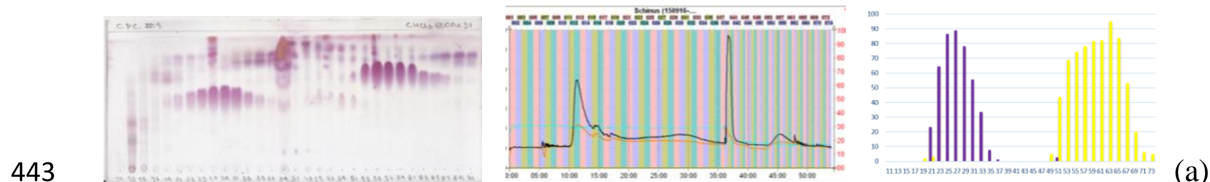
429 Explanation of Kd shifting in **Figure 2** when compared to Parts I and II:

Equipment used	Published information	Place of separation	Kd Value 3-β-masticadienolic acid
Mini DE	Part I	London, UK	0.8
Spectrum DE	Part I	London, UK	0.7
Midi DE	Part I	London, UK	0.8
Spectrum DE	Part II	Braunschweig, Germany	1.2
Pharma Tech CCC 1000	Part II	Braunschweig, Germany	1.0
Quattro HT-Prep CCC	Part II	Rio de Janeiro, Brasil	0.7
Coil Separator PC Inc	Part II	Rio de Janeiro, Brasil	0.8
ASCPC250 – Part III	Part III	Reims, France	2.2

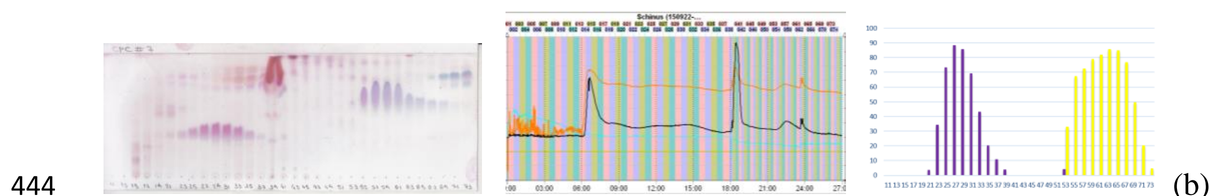
430

431 The same portion of crude material was used for all 3 papers, which was prepared in
432 July 2012. TLC plates for the crude material still look exactly the same as 4 years ago.
433 We know from our experience, which has been confirmed by PhD work carried out at
434 Brunel University London in 2012-2015 [Reference 15 on manuscript], that Kd values
435 of ionised compounds might change if their pKa is close to the pH of a solvent system
436 used.
437 In the case of HEMWat pH is 5.5 and pKa of the 3-beta-masticadienolic acid is 4.81
438 (according to ChemAxon Physico-chemical property predictors,
439 https://www.chemaxon.com/), which means that we would have different degree of

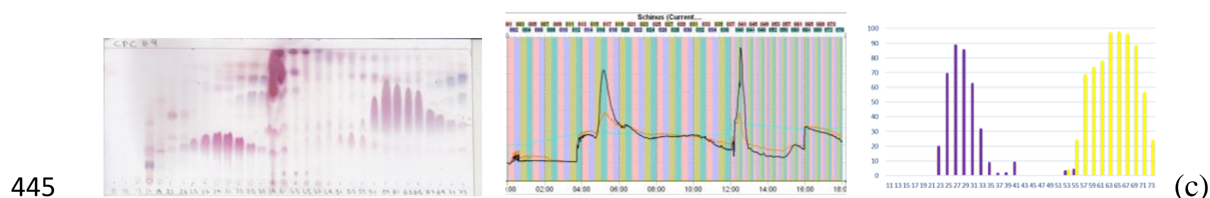
440 ionisation depending on residence time of compound in the solvent system and its
441 concentration.
442



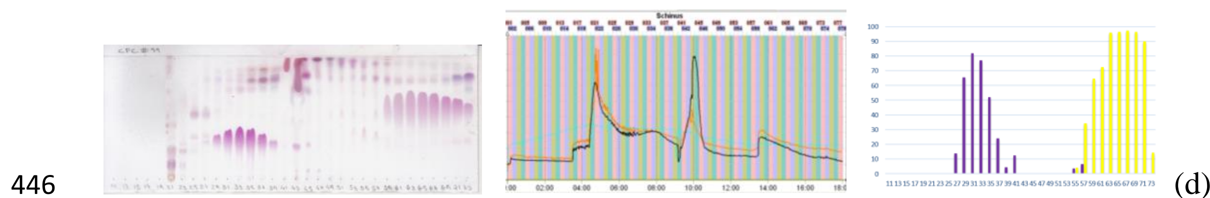
443 (a)



444 (b)

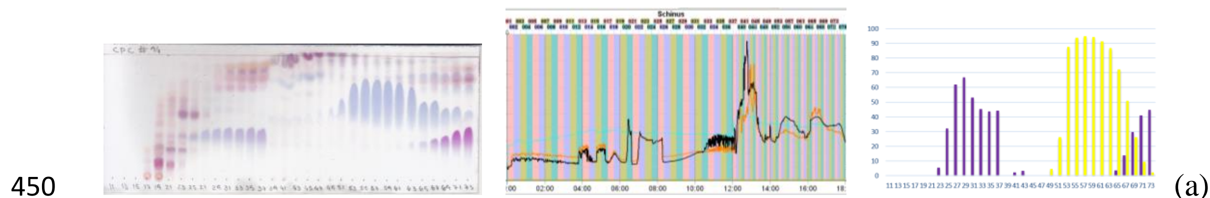


445 (c)

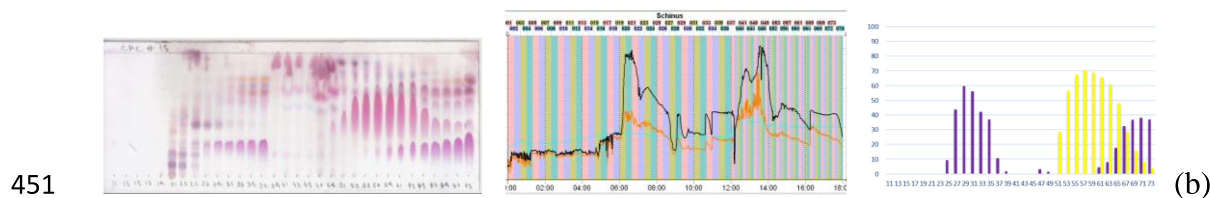


446 (d)

447 **Figure S1.** TLC, CCC chromatogram (254 nm) and fractogram of CPC flow-rate
448 experiments at (a) 7 mL.min^{-1}, (b) 14 mL.min^{-1}, (c) 21 mL.min^{-1}and (d) 28 mL.min^{-1}.
449

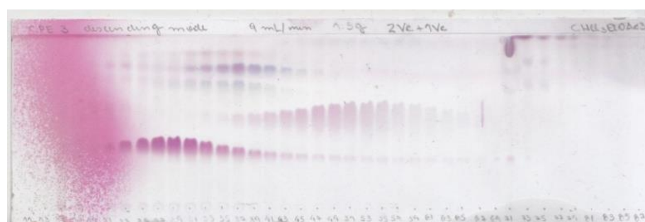
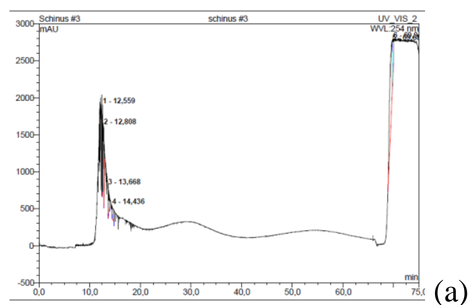


450 (a)



451 (b)

452 **Figure S2.** TLC, CCC chromatogram (254 nm) and fractogram of CPC sample volume
453 experiments at (a) 24 mL and (b) 35 mL.
454
455

456  

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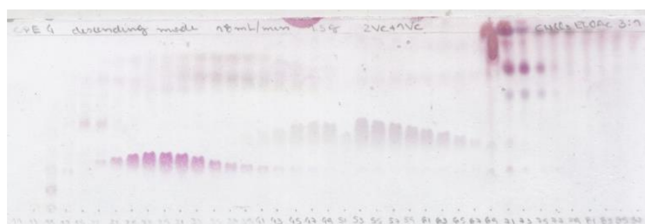
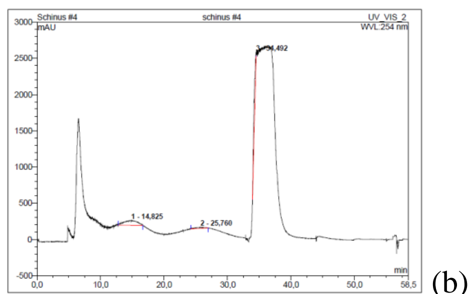
457  

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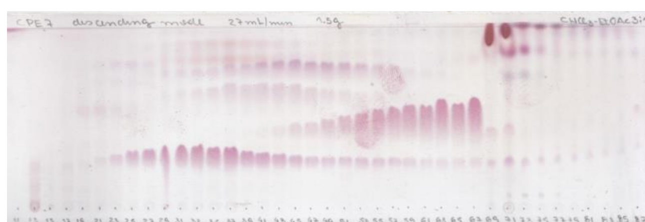
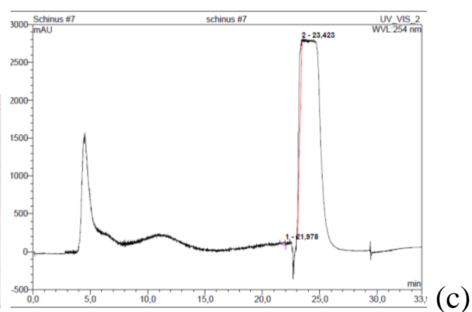
458  

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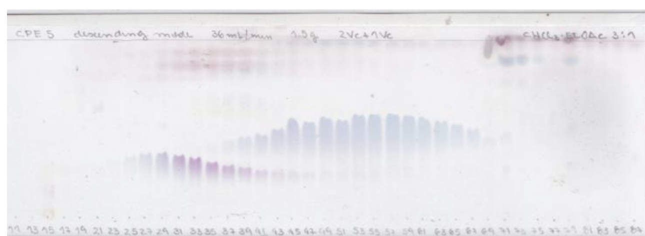
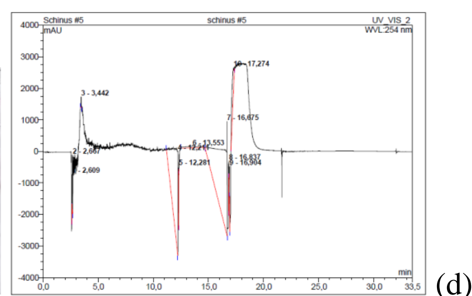
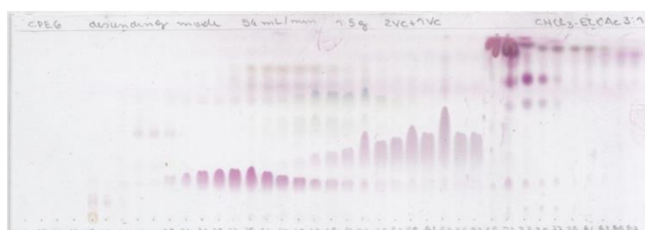
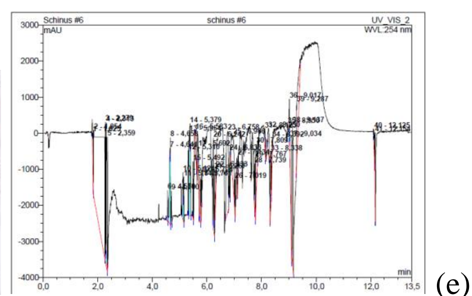
459  

(d)

460  

(e)

461

462 **Figure S3.** TLC and CCC chromatogram (at 254 nm) of CPE flow-rate experiments (a)
463 9 mL.min^{-1}, (b) 18 mL.min^{-1}, (c) 27 mL.min^{-1}, (d) 36 mL.min^{-1} and (e) 54 mL.min^{-1}.

464