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# *Schinus terebinthifolius* countercurrent chromatography (Part III): Method transfer from small countercurrent chromatography column to preparative centrifugal partition chromatography ones as a part of method development☆

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ABSTRACT

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



## 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force (droplet countercurrent chromatography – DCCC – and rotational locular countercurrent chromatography – RLCC) have been replaced by more efficient equipment which uses centrifugal force to hold the stationary liquid phase [1].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

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**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 |
| $\overline{h_{cell}}$ (cm) | – | 0.69 | 1.45 |
| *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 ($mg.mL^{-1}$)[a] | 4.88[b] | 11.96 | 13.04 |

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

**Table 2**
Experimental details.

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

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

## 2. Experimental

### 2.1. Materials

All solvents – Heptane (Hep), ethyl acetate (EtOAc), methanol (MeOH), acetonitrile ($CH_3CN$) – were purchased from Carlo Erba Reactifs SDS (Val de Reuil, France). Deionized water was used to prepare aqueous solutions.

*Schinus terebinthifolius* berries dichloromethane extract, solvent system and sample preparation methodology was taken from a previous work [11].

### 2.2. CCC and CPC instruments

Three support-free liquid-liquid instruments were used in this work:

Mini DE centrifuge (Dynamic Extractions, Tredegar, UK) equipped with a polytetrafluorethylene (PTFE) multi-layer column (17.4 mL and 0.8 mm i.d.). The distance between the central rotor axis and the column axis is 50 mm. The β-value ranges from 0.50 to 0.76 and the rotation speed is adjustable from 200 to 2100 rpm producing *g* field reaching 500 g level at periphery of the column. The system is equipped with an Agilent HP1100 (Santa Clara, California, U.S.A.) pump and a Foxy Jr, Teledyne Isco (Lincoln, Nebraska, U.S.A.) fraction collector.

The FCPE300 device (Kromaton Technology, Angers, France) was equipped with a rotor of 7 stacked partition discs engraved with a total of 231 twin partition cells. The total volume of the column is 303 mL and the volume of interconnecting cell ducts is 73 mL. The rotation speed can be adjusted from 500 to 2000 rpm, producing a relative centrifugal acceleration in the partition cell up to 437*g*. Phases were pumped with a KNAUER Preparative Pump 1800 V7115 (Berlin, Germany). The system was coupled to a UVD 170S detector (Dionex, Sunnivale, CA, USA) equipped with a preparative flow cell. The eluent was monitored at 254 nm. Samples were injected through a sample loop with volume varied according to Tables 1 and 2. Fractions were collected by a Pharmacia Superfrac collector (Uppsala, Sweden). Chromatographic data were acquired by using the Chromeleon Software version 6.11 (Dionex).

The CPC ASCPC250 (Armen Instrument, Vannes, France) was equipped with a 250 mL rotor containing 21 stacked discs with a total of 1890 twin-cells was used. The total active volume is 214 mL (about 0.1 mL per cell) and the volume of interconnecting cell ducts is 30 mL. Rotation speed could be adjusted from 500 to 3000 rpm, thus producing a centrifugal force field in the partition cells up to 700 *g*. Samples were injected through a sample loop. The solvents were pumped through a semi-preparative 4-way binary high-pressure gradient Armen Light version pump (50 mL/min maximum flow-rate, 150 bar). The detection was done by UV Armen Detector at 254 nm. Fractions were collected by