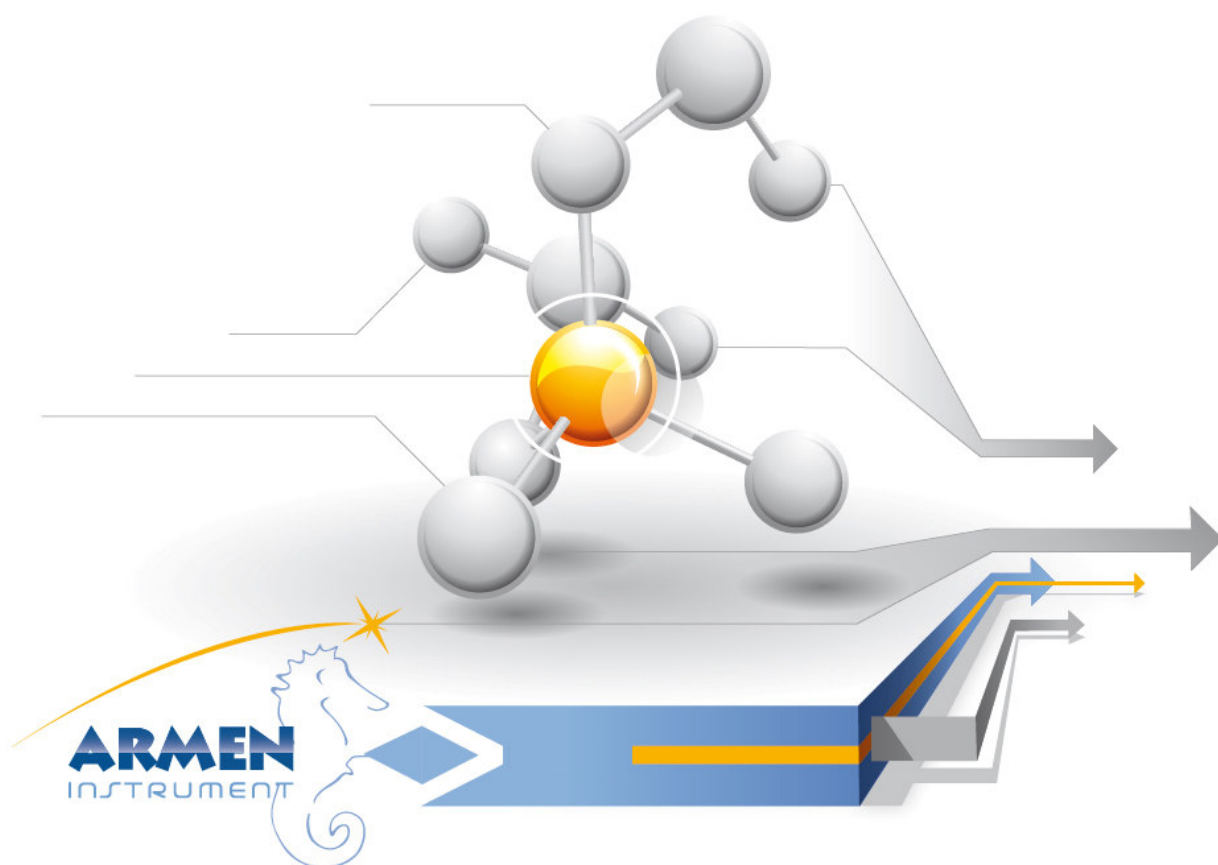


INTRODUCTION TO CENTRIFUGAL PARTITION CHROMATOGRAPHY CPC/CCC



Specialist in preparative and industrial LC

What is Centrifugal Partition Chromatography or CounterCurrent Chromatography?

Centrifugal Partition Chromatography (CPC) also known as Counter Current Chromatography (CCC) is a preparative, pilot and industrial liquid purification technique that does not require traditional solid supports. The main aims of this technology are to isolate the maximum amount of a specific molecule at the highest purity, in a minimum of time and without using any silica column or support media.

CPC/CCC and prep HPLC do have some similarities:

- Same objectives
- Same fundamental chromatographic process,
- Identical peripherals : pumps, injectors, inline detectors and fraction collectors.

The heart of LC instrument in general is the column where the separation occurs. The fundamental difference between LC like flash or HPLC and CPC/CCC is the nature of the stationary phase.



The stationary phase in classical LC is made of coated or non-coated silica where the skeleton of the particle is only a support and the surface generate chemical interaction with mobile phase and molecules to be separate.

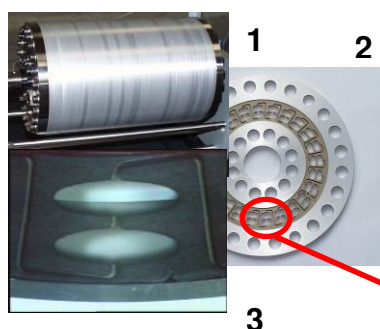
Centrifugal Partition Chromatography does not require a solid support like silica: two non-miscible phases used, one as the mobile phase or the eluent and the other as the stationary phase maintained by the centrifugal field. The affinity of the solute for each phase can be measured by their partition coefficient that in turn dictates the order of elution for each compound.



How to maintain liquid stationary phase inside a LC column?

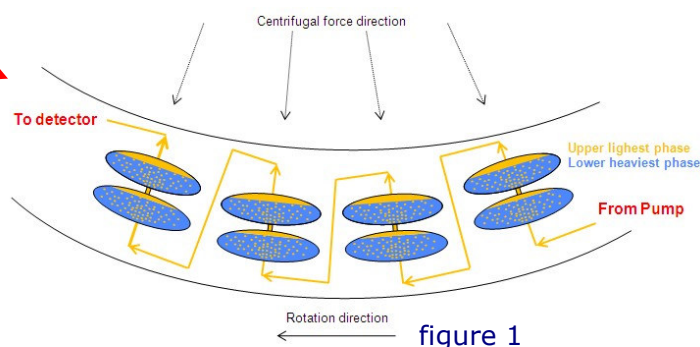
Classical LC columns are simply made: a cylinder (stainless steel in HPLC and plastic or glass in flash chromatography) with inlet and outlet at each extremity, filled with silica particle of difference size according to the efficiency required and pressure resistance.

The CPC/CCC column also has an inlet and outlet for the mobile phase, but these are the only similarities with an HPLC column. To be able to maintain one phase of the biphasic system inside the column, a centrifugal field and special design must be done.



CPC/CCC are made with one axis where one rotor or column is installed (picture 1). At each extremity of the column, a rotary seal allows the passage of the liquid from the static to the rotating part. The column is made up of stacked disks (picture 2) where more than a thousand cells are linked together by a thin engraved duct (picture 3). The rotor or column is kept under a homogenous centrifugal field which allows retention of the stationary phase thanks to the specific geometry of the cells (picture 3).

A four way valve allows a change in the direction of the elution and therefore will work either in ascending mode (figure 1) when the lightest phase is the mobile phase or in descending mode when the heaviest phase is the mobile phase. By working this way, it is possible to work both in normal and reversed mode without replacing the column.



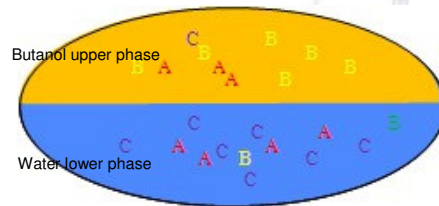
ASCENDING MODE

How separation occurred inside Armen SCPC column?

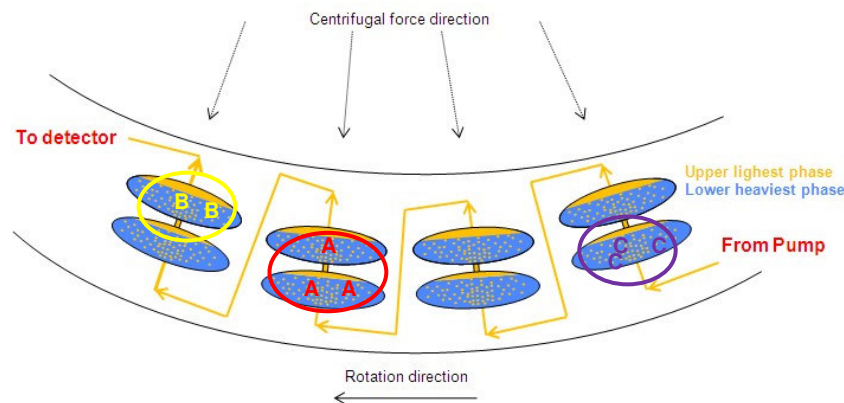
Chromatographic effect in CPC/CCC can be easily explained. Place two non miscible solvent (for example butanol and water) in a separatory funnel, shake and wait for equilibrium: the upper phase is made of a majority of butanol and lower phase of a majority of water. After dissolution of a molecule A with a concentration [A] in 2 mL of biphasique system made with 1 ml of each phase, shaking and equilibrium (two phases), A is more or less dissolves in both phases. At the equilibrium, for a given temperature, the concentration ratio (respectively [A]_{upper} and [A]_{lower}, [A]=[A]_{upper}+ [A]_{lower}) allows us to define the partition coefficient K_d :

$$K_d = \frac{[A]_{\text{stat}}}{[A]_{\text{mob}}}$$

From this it results that for a K_d close to 0, the molecule is completely dissolved in the mobile phase and will not be retained in the system. For a K_d that is too high, the molecule will be too well retained in the stationary phase. So, the best partition coefficient for a good separation is between 0.5 and 5. The Solvent system can then be determined according to the partition coefficients of all molecules that need to be separated.



$$\begin{aligned} K_{dA} &= 1 \\ K_{dB} &< 1 \\ K_{dC} &> 1 \end{aligned}$$



Choosing solvent system in CPC/CCC is like choosing the column and the eluent in HPLC

What are the advantages of CPC?

The volume of stationary phase available (more than 60 %) and the absence of a silica means that there are a number of advantages for this technique versus traditional separation methods.

- ☞ No column to replace, no silica to recycle
- ☞ Low solvent consumption
- ☞ High flow rate for low run time
- ☞ High performances. Purity > 99%, recovery > 90%
- ☞ No sample losses
- ☞ No denaturation, no irreversible adsorption of the sample
- ☞ Huge application fields from petroleum extract to proteins.

CPC/CCC could be a good alternative of classical LC purification. It could be used as a pre-purification of really complex extracts (such as natural products or biological extracts) but also for the final purification step to obtained pure compounds.

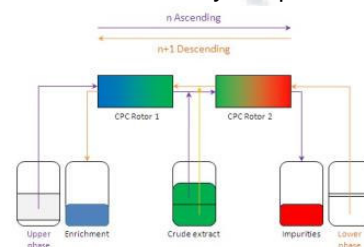
Moreover as there are no unwanted secondary interactions with silanols on the silica, this technique therefore preserves the integrity of fragile compound without denaturation or losses by irreversible absorption

What is Armen True Moving Bed CPC?

In addition to batch SCPC instruments, Armen Instrument proposes also a complete range of continuous SCPC systems using TMB concept.

Separation in CPC is generally done by injecting a defined volume of sample at one extremity of the column. Armen TMB systems are made of two CPC columns with four pumps. The process could be easily compared to SMB as Simulated Moving Bed used in HPLC for continuous injection.

Injection of the sample is done continuously between both columns and the separation occurred by sequence of two steps whereby the mobile phase is alternatively the upper phase and the lower phase. For a simple binary mixture it means that the molecule that has more affinity for lower phase will be elute on the left of the system and the molecule that as more affinity for the upper phase will be elute on the right part.



Here is presented a continuous separation of two dyes. The green mixtures is continuously injected between both columns, pure red dyes is recover in the right outlet of the system and blue one in the left part.

The difference with a simple biphasic liquid extraction system in funnels is that CPC TMB gives enough separating power in terms of plate efficiency to separate very similar molecule as diastereoisomer. The size of the CPC TMB columns defines the scale of productivity, for example a 12.5 litre system is capable of delivering kg scale levels of compound and larger systems can further enhance these levels.

When can we use SCPC TMB systems?

SCPC TMB could be used as continuous and high efficiency liquid liquid extractor for the enrichment of natural extracts and to eliminate non-desirable molecules, or in a binary mixture to eliminate molecules of secondary importance, or to concentrate a low content molecule in a crude mixture. Armen Instrument has a number of application notes on a subject that can be downloaded from our web site

Where can I test SCPC or TMB and which services do Armen provide around this technique

Armen Instrument has an extensive range of services that makes CPC an easy to use technology. Our fully equipped application laboratory and collaborations allows us to propose:

- 🔗 Custom training on CPC technology
- 🔗 Demonstration
- 🔗 Services for purification: feasibility study, scale up and production
- 🔗 Complete database for your method development

Equipment installed in our laboratory:

- 🔗 SCPC100 for method development
- 🔗 SCPC1000 for scale up
- 🔗 SCPC12,5L for production
- 🔗 TMB250
- 🔗 Armen Spot flash II system
- 🔗 Armen Spot prep II system
- 🔗 LaChrom HPLC with Diode Array and auto sampler
- 🔗 Agilent GC system with autosampler
- 🔗 Buchi rotavap 1L and 20L capacity
- 🔗 GMP environment fully

Armen SCPC line of products: easy to use integrated solution

Armen instrument has been developing robust and reliable prep & industrial LC system for over 20 years. The Armen SCPC systems represent the latest advances in CPC/CCC technology and have made this unknown technology popular, easy to use and reliable for semi prep, prep and industrial purification.



ARMEN SCPC-12.5L



ARMEN SCPC-250

Armen instrument has a complete range of CPC systems with various column capacities; 100 ml, 250 ml, 1L, 5L, 12.5L and custom industrial systems.

Our SCPC-100+1000 or SCPC-250+1000 systems integrate small columns for method development or multi mg purification and the 1L column for multigram injection in one box. Working from one to the other column is possible by a simple switching column valves.



From simple SCPC column to fully integrated and automated all our SCPC columns could be connected with the Armen LS5600 fraction collector, Armen AP pumps, injector and all kind of detectors.

Coupled with a Spot prep II system our SCPC column will allow you to perform prep HPLC and CPC runs by switching from one column to the other.



Armen SCPC range

Reference	Column volume	Max. rotation speed	Typical Flow rate	Injection range
SCPC-100	100 mL	3000 rpm	10-20 mL/min	From mg to g
SCPC-250	250 mL	3000 rpm	10-20 mL/min	From mg to 5 g
SCPC-1000	1 L	3000 rpm	20-50 mL/min	15-30 g
SCPC-100+1000	100 mL + 1L	3000 rpm	20-50 mL/min	3-6 g & 15-30 g
SCPC-250+1000	250 mL + 1L	3000 rpm	20-50 mL/min	3-6 g & 15-30 g
SCPC-5000	5 L	1500 rpm	150-200 mL/min	100-150 g
SCPC-12.5	12,5 L	1500 rpm	1.5 L/min	# 1 kg
Custom SCPC	Up to 50L			
TCPC-250 TMB ^{TM*}	2 x 125 mL	3000 rpm	10-20 mL/min	
TCPC-1000 TMB ^{TM*}	2 x 500 mL	3000 rpm	20-50 mL/min	
TCPC-12.5 TMB ^{TM*}	2 x 6.25L	1500 rpm	1.5 L/min	Continuous
Custom TCPC	Up to 50L			

Armen peripherals for SCPC column

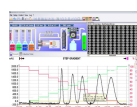
Spot prep II : Complete prep HPLC system that could be connecting to classical LC column and CPC column. Pump, automated loop injector, UV/Vis DAD600, fraction collector, touch screen PC with software AGCPC all integrated in one module



Fraction collector LS5600 :

Pump

AP50 pump : 50 ml/mn, 300 bars Isocratic, binary or quaternary gradient
AP100 pump : 100 ml/mn, 250 bars Isocratic, binary or quaternary gradient
AP250 pump : 250 ml/mn, 230 bars Isocratic, binary or quaternary gradient
AP500 pump : 500 ml/mn, 110 bars Isocratic, binary or quaternary gradient
AP1000 pump : 1L/mn, 80 bars, bars Isocratic, binary or quaternary gradient



Armen Glider software

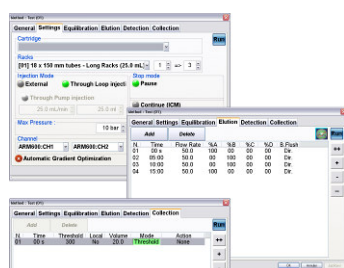
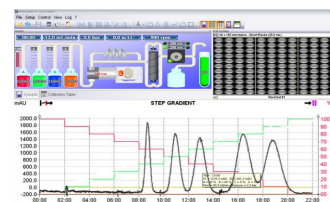
Detector : Armen UV/Vis DAD 600 with prep flow cell



Download PDF brochure on this entire product on our web site

Armen Glider CPC software

In addition to the development of enhanced CPC and prep LC hardware, Armen instrument develops software solutions designed to meet your specific needs. The Armen Glider Software (AGS) for prep LC and CPC is specially optimized for simple and intuitive access. All peripherals are under single point control from the CPC rotation to fraction collector, detector ...



With AGS, you develop your complete method from stationary phase loading, equilibration, injection, elution, extrusion or dual mode, put the solvent bottle in the right place and performed your run with one click.

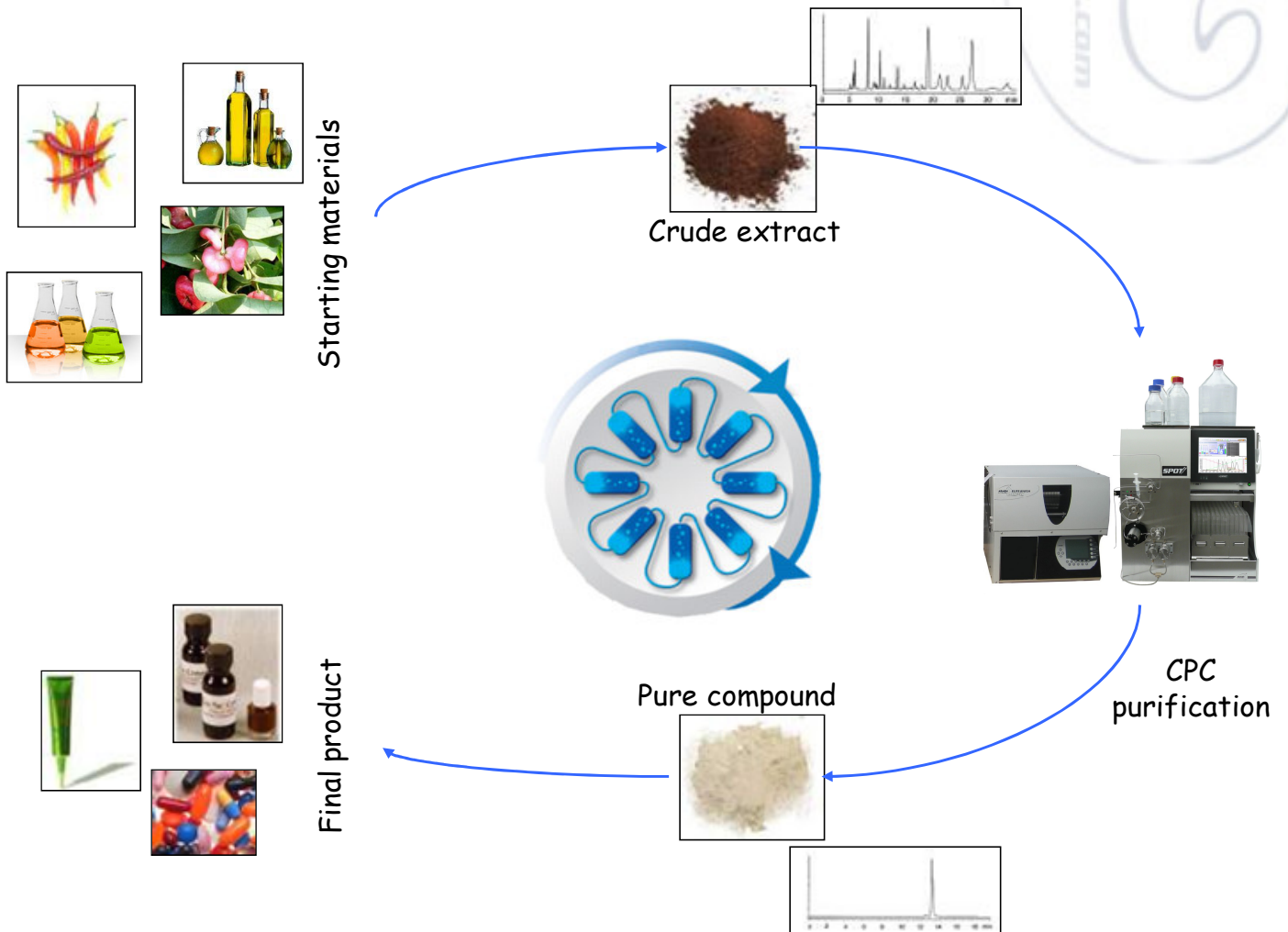
Collection of your fractions can be defined according to time, volume, threshold and peak. Methods can also be modified during optimization runs

Armen instrument provides a global solution for your purification project.

Complete range of preparative to industrial LC system (prep HPLC and CPC)
Simple and intuitive software for automation and easy access
Services for purification : from feasibility studies to production

www.armen-instrument.com

ARMEN CENTRIFUGAL PARTITION CHROMATOGRAPH SYSTEM



SOLUTION FOR PURIFICATION