Automated Method Development Strategy for Countercurrent Chromatography

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Introduction

Countercurrent chromatography (CCC) is a liquid-liquid separation technique. The pairing of two immiscible liquids, one as stationary phase (SP) and the other as mobile phase (MP), effects a separation of the components of a sample through a single partitioning mechanism. The SP is first pumped into the column, typically a length of plastic tubing mounted on a rotor which is spun rapidly. This high speed rotation results in a centrifugal force which is a key parameter for retention of the liquid SP. The MP is then pumped through the column, partially displacing the SP, until equilibrium is reached between the two phases (figure 1).

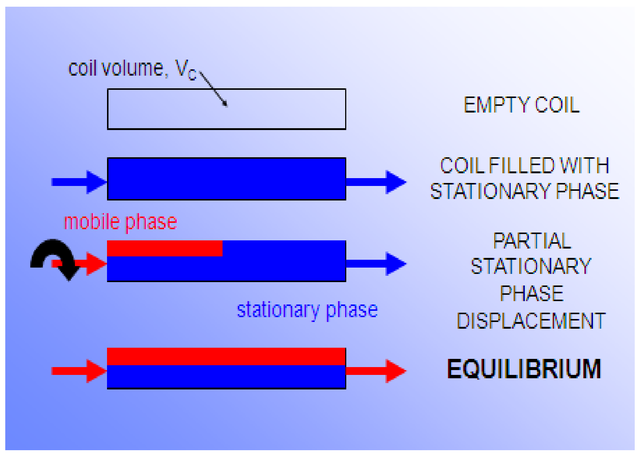


Figure 1. CCC Process

Once equilibrium is established, sample is injected into the column and separation of the its components takes place via repeated partitioning between SP and MP during the transit of the MP through the column.

CCC is a purification centric technique based on these traits:

- High loading - large volume of stationary phase
- Full recovery - no irreversible adsorption of the solutes in the liquid stationary phase
- Predictable scale-up - retention based on single mechanism of separation – partitioning
- Separation based on selectivity
- Large array of possible solvent systems provides a wide spectrum of selectivity

The large number of possible solvent choices can make the selection of the appropriate solvent system a difficult task. Selection of the best solvent system is essential for a successful purification. We propose a methodical strategy, with the aid of an automated screening system, to reduce the time and effort required for such a task.

Experimental

The automated CCC screening system (Figure 2) consists of:

- Agilent pump (G1311A), degasser (G1322A) and UV detector (G1315B)
- Dynamic Extractions Spectrum HSCCC (fitted with a 24 ml.column)
- PDR-Chemical AutoCCC modules (10-port solvent mixer, injector/fraction collector and switching valves)

System controlled by PDR-Chemical AutoCCC software version 1.1

The traditional manual system consisted of:

Agilent pump (G1311A), degasser (G1322A), autosampler (G1313A) and UV detector (G1315B)

Dynamic Extractions Spectrum HSCCC (fitted with a 24 ml.column)

System controlled by Agilent Chemstation software version B.03.02

Comparison of the two systems was done using a solution of test probes run in triplicate. Details of the chromatographic runs are given in figure 3.

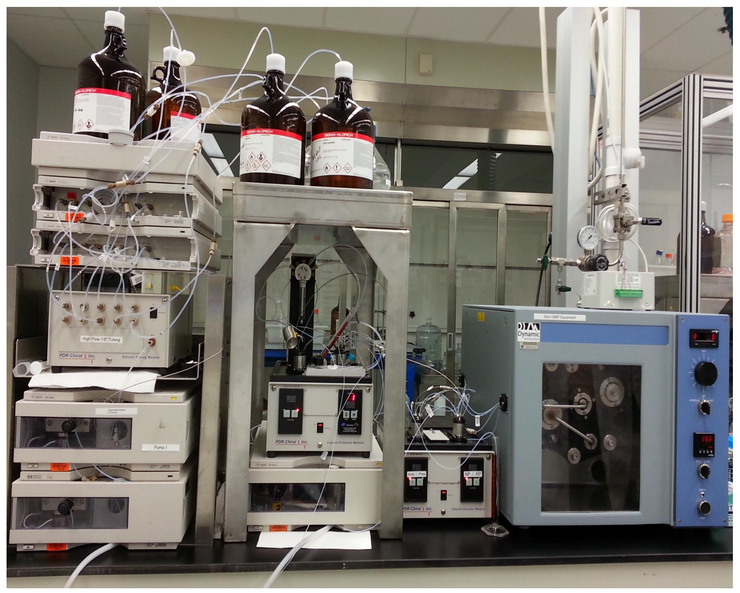


Figure 2. Automated CCC system configuration

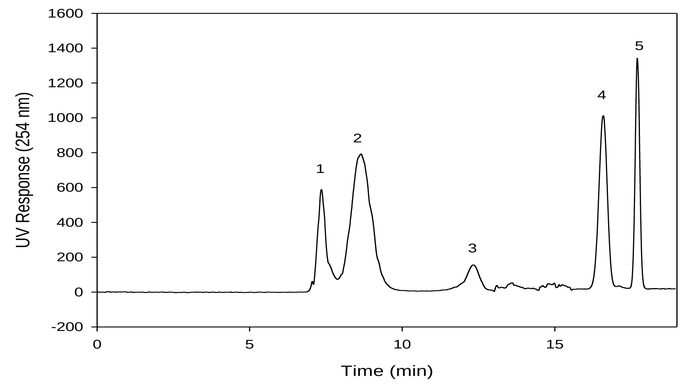
Discussion

Because of the wide range of solvent combinations available for use in CCC, method development can be very time consuming. The purpose of the automated screening system described here is to reduce the time and effort involved in this task. We focused on four solvent system sets, which have overlapping polarity ranges and complementary solubility properties. Each set is comprised of solvent blends with several different ratios.

Set 1 heptane/ethyl acetate/methanol/water (5 solvent ratios)
Set 2 dichloromethane/methanol/water (3 solvent ratios)
Set 3 methyl-t-butyl ether/acetonitrile/water (3 solvent ratios)
Set 4 heptane/toluene/acetone/water (3 solvent ratios)

To establish a baseline we screened Set 1 using the traditional manual system described above. Figure 3 is the representative chromatogram from this screening effort. As this approach is not continuous and with the long idle time in between sets/overnight, it would take over three days to screen all four solvent sets in this manner.

We then screened Set 1 using the automated system and obtained similar chromatography. The key difference compared to the manual system is the addition of a ten-port solvent mixer. We then screened all four solvent systems once with water and again with 0.1% aqueous TFA. Unlike the manual process, this approach was continuous with no operator intervention needed to change solvents and, more significantly, no idle time overnight. It took less than a day to perform the screening.



Solvent system :heptane/ethyl acetate/methanol/water in 1:1:1:1 ratio with 0.1% trifluoroacetic acid run in elution/extrusion mode with upper phase as SP and lower phase as MP. Column diameter /volume :0.8 mm /24 ml. CCC temperature controlled at 30°C. 1) Dipyrimidamole, 2) 4-Bromobenzamide, 3) Warfarin, 4) Methyl-2-acetamido-5-bromobenzoate 5) Biphenyl.

Figure 3 Representative CCC Chromatogram

The common CCC solvent screening practice is to use a quaternary HPLC pump that can only mix a maximum of four solvents at a time. This approach is inefficient when more than one solvent system and/or additives in the aqueous phase need to be assessed. However, with our automated configuration, greatly expanded solvent blending possibilities are realized. This allows continuous unattended screening of multiple solvent system sets and results in significant efficiency gains during method development. Table 1 shows a comparison of the two approaches.

Automated					Manual				
	Cycle time (hr)	# cycles	runtime (hr)	runtime (day)		Cycle time (hr)	# cycles	runtime (hr)	runtime (day)
one pass	0.75	14	10.5	0.44	manual day 1	0.75	14	10.5	0.44
repeat w/ pH adjust	0.75	14	10.5	0.44	idle time at night			16	0.67
total			21	0.88	manual day 2	0.75	14	10.5	0.44
					sub-total			37	1.55
					repeat w/ pH adjust			37	1.55
					total			74	3.60

Table 1 Comparison of screening times – automated vs. manual

Conclusion

For CCC to gain traction in pharmaceutical applications, the method development process needs to be more efficient compared with other solid-phase purification techniques. The automated system described here allows 24/7 operation providing a greater than three-fold time savings compared to a typical CCC method scouting process. There are many other solvent systems we did not consider and only focused on four sets in our development strategy. The solvent system selection is key to a successful purification. We will continue to assess other solvent systems to further optimize this screening tool.