Scale-up in drug development

High Performance Counter Current Chromatography can be very useful to drug developers when scale-up or sample solubility cause purification problems. **David Keay** of Dynamic Extraction explains why

Pharmaceutical companies are developing compounds of increasing complexity, drugs with numerous functional groups on a single molecule that must be purified to standards that are ever more stringent. Liquid chromatography (HPLC) has been added to the classical method of crystallisation to broaden purification capabilities, but there is still an acute need for further tools to address the growing number of purification problems.

These problems normally manifest themselves in one of two ways. The first is the solubility limits of the sample to be purified, which restricts the loading that can be achieved and greatly influences the overall process time that any purification takes to perform. The second is the scale-up of purification processes, especially with HPLC, which are complicated tasks that often require redeveloping as scale is increased. Therefore, chemists spend most of their time conducting chromatography development, rather than being able to focus their efforts on drug development.

This redevelopment consumes precious time and, considering the attrition rate of drug candidates in the development process, is ultimately wasted. Hence, reducing it is a key goal. In a perfect world the solution would be simple – a purification technique with a high capacity that is easily scaled once the purification method is developed and optimised.

Fantasy possibly, but there is now a technique that has demonstrated both these capabilities from the analytical through to the kilo scale. The pharmaceutical industry is accepting this new technology, called High Performance Countercurrent Chromatography (HPCCC), because instruments are now available for a wide range of scales from analytical to production.

immiscible liquids

HPCCC uses a liquid, rather than solid, stationary phase to perform chromatographic separations. The technique uses immiscible liquids for both mobile and stationary phases, with simple partitioning being the mechanism of purification.

Partitioning is a simple process to understand, since it depends only on the partition coefficient of a target molecule between two immiscible solvents. With only one mechanism of purification, scale-up from analytical through preparative to kilo scale becomes easy.

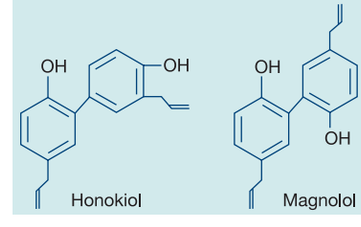


Figure 1: Top - Dynamic Extraction can provide a full rangeof HPCCC instruments
Figure 2: Above -The optical isomers Honokiol and Magnolol

The use of a liquid stationary phase provides access to the entire volume of the stationary phase rather than just the surface, as is the case with solid phase techniques, such as SPE or HPLC. This allows higher sample loadings, total sample recovery, no irreversible adsorption of target compound, and new operating strategies can be used.

HPCCC instruments should be considered as a new type of column that makes liquid stationary phases available for high-resolution liquid chromatography applications across a broad range of molecule types from natural to synthetics. These columns are simply placed into existing liquid chromatography systems. Very quickly scientists can evaluate the benefits these columns can offer their purifications and, in particular, decide whether they can help solve their sample solubility or scale-up challenges.

However, it is only with the ▶

Table 1: Comparison of purification data for the separation of optical isomers honokiol and magnolol

	HPCCC	HPLC
Stationary phase	Lower phase of hexane-ethyl acetate-methanol-water (1:0.4:1:0.4, v/v)	Zorbax Eclipse XDB-C18 column 250x9.4mm ID 5 μm
Mobile phase	Upper phase	Methanol-water (70:30, v/v)
Sample capacity per run (g)	43	1.96×10^{-2}
Run time min	45	40
Productivity mg/min	431	0.49
Purity of isolated compounds	>99.9%	>99.0%
Solvent consumption (L/g)	1.39	5.10

advent of analytical HPCCC instruments that the technique can be applied to a full range of applications throughout the medicinal chemistry field. Dynamic Extractions is currently the only company that provides the full range of HPCCC instruments allowing purifications with sample injection masses of milligrams through to kilos. Now chemists have to commit only a fraction of their available material to the purification development and know that once the development has been performed, the developed method can be used through to the kilo scale and beyond.

solubility challenge

Sample solubility is often the rate-determining factor of any purification using a solid stationary phase. The reason is that in any solid stationary phase technique, the only way to introduce the sample is in the mobile phase. The advantage of HPCCC is that the sample can be dissolved in either the mobile or the stationary phase with no impact on the quality of purification.

The impact of HPCCC on solubility challenges can be dramatic. An example is the comparison of purification data for the separation of two optical isomers, Honokiol and Magnolol (the structures of these molecules are shown in Figure 2). Using HPLC to perform anything other than milligram purifications is a very time-consuming process. The use of HPCCC has enhanced the process, as shown in table 1. In productivity terms, HPCCC has improved the process by a factor of 1000.

drug development

The ideal separation technology for industry is one that allows direct scale-up. Put simply, the same separation can be obtained at any scale. A separation is defined in terms of purity, yield and time: higher productivity is achieved by separating greater amounts of sample in the same time with identical purities and yields producing matching chromatograms.

In solid phase separations, such as HPLC, columns of a standard length are used. Enlarging the cross-sectional area of the column increases the column volume. Changing the sample volume and mobile phase flow rate in proportion to the column volume allows the same separation to be reproduced in any size of column and scale. However, because at the analytical scale the stationary phase used has to be very small beads, as the process is scaled up the pressure drop across the column rises dramatically, typically beyond the mechanical limits of the column. Therefore, the separation has to be redeveloped using the same material, but with a larger bead size.

This change affects several other variables, which has an impact on the resolution of the purification and, therefore, considerable time is spent in redevelopment to replicate the original analytical purification at a larger scale. Every time the scale is increased, it is likely that the chromatography will require redevelopment again.

However, this problem with the stationary phase does not arise with HPCCC. Larger quantities are freely available, simple to prepare in a larger scale column and with little overall increase in pressure drop of the system. This means that the same operating conditions can be easily created at larger scales and the purification straightforwardly replicated at the larger scale. This allows chemists to focus on their true role, drug development, rather than wasting time on chromatography development.

It has been demonstrated using HPCCC instruments that scaling, once the method is developed and optimised, is solely a matter of the scaling by the volumetric ratios of the column volumes. Therefore, if an analytical purification is performed on a 20ml column and the preparative scale column is 1,000ml column, then the sample volume and the mobile phase flow rate are simply multiplied by a factor of 50. Figure 3 shows two examples of analytical to preparative scale separations and below is a comparison of the chromatograms of an analytical to a pilot scale purification.

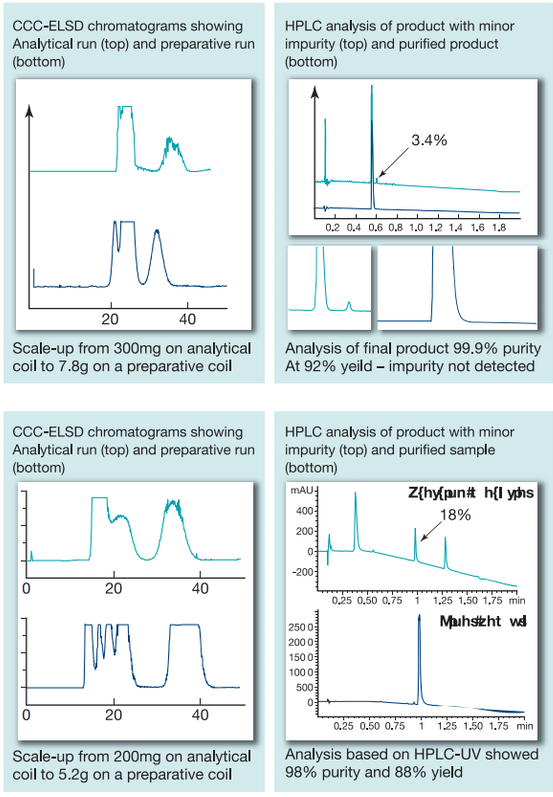
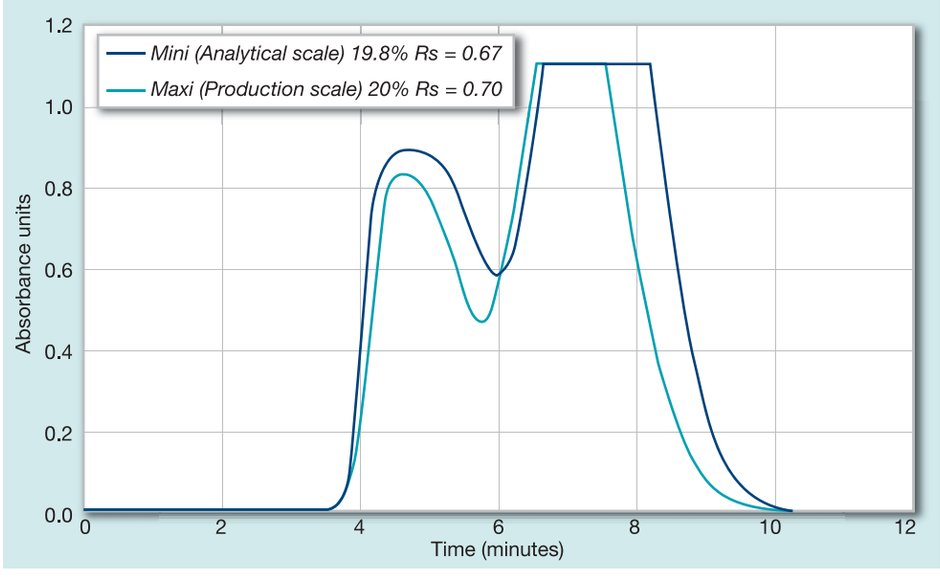


Figure 3: Top - Scaling up from analytical to semi-preparative - minor impurity removal
Above - Scaling up from analytical to semi-preparative - impurity isolation for indentification purposes

elution strategies

These days there are many new ways of operating solid phase techniques – low viscosity mobile phases (SFC), ultra high-pressure liquid chromatography (UHLC) or multi-column chromatography (SMB), but all are just variations where a particular variable is altered and none are fundamental or disruptive changes. Hence, only a step change in performance is produced.

When using HPCCC the possibility exists for fundamental change. The reason for this is that since both ▶



Comparison of the chromatograms of an analytical to a pilot scale purification

New Elution Strategies

Elution Extrusion – This strategy makes use of the fact that compounds may be fully separated inside the column before eluting. Due to the fact we are using a liquid stationary phase, we are able to recover the separated compounds without completing the full elution cycle. In elution extrusion, the separation is started in the same manner as in single-mode CCC. However, when the run reaches a certain point the mobile phase is stopped and stationary phase pumped in to extrude the column contents. This enables the purification cycle and solvent usage to be significantly shortened and the column after extrusion is new and ready for the next injection.

Dual Mode Elution – When operating a dual mode elution operating strategy, the aqueous phase is first pumped as the mobile phase (i.e. normal phase operation) and after a set period of time the organic phase is then pumped as the mobile phase (i.e. reverse phase operation). This switching procedure can take place a number of times until the desired resolution of purification is achieved. The advantage of this method is that compounds having strong affinity for the original stationary phase can also be separated quickly, rather than waiting a long time for them to elute in the mobile phase.

pH Zone Refining - This strategy uses the phenomenon that charged entities (ions) prefer the aqueous phases and uncharged molecules prefer organic phases and the strategy employs basic organic phases and acidic aqueous phases (or vice versa). The analytes dissolved in the stationary phase are eluted by mobile phase according to their pKa values and solubility. For these types of molecules, it enables very large loading capacity and high-resolution separations to occur.

◀ mobile and stationary phases are liquid in these columns both can move either independently or together. This gives new operating options that are impossible with systems that use solid stationary phases.

These new options can be used to reduce overall processing time, dramatically reduce solvent consumption and provide a purification solution that technologies where only the mobile phase is flowing could not. The panel above describes a number of these strategies in more detail.

However, there are more developments that are currently being researched. In the near future using this type of technology true moving bed (TMB) chromatography and continuous processing options will become practical approaches that offer significant capital and operating cost benefits to users.

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conclusions

Now that High Performance CCC instruments have become available the pharmaceutical industry is keen to evaluate the benefit that the technology offers. Key to this has been the introduction of analytical scale equipment that allows purifications to be developed on milligrams of material. This is key because it allows development and optimisation at the milligram sample injection scale, knowing that scale-up to gram and kilo scale is simple.

The technique is providing an additional tool to chemists to help them solve current purification challenges. High Perfomance CCC has been found to be particularly useful where scale-up or sample solubility or crude sample condition are causing purification problems with synthetic, macro and natural molecules. ■

acknowledgement
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