

A New Tool for Purification

HPCCC as an Alternative Liquid Chromatography Technology

Pharmaceutical companies are looking for ways to increase the processing speed of developing new products and at the same time reducing the ever increasing costs of this process. This cost is a function of the large number of potential candidates, each requiring the investment of money and resource, when all but a one or two will fall by the wayside.



The main cause of this waste is that for each candidate that makes it to the final hurdle of phase III trials, as many as 10,000 will have been tested and discarded. Therefore chemists are now looking for processes that can purify crude samples without investment in developing the chemistry of each candidate, while still enabling toxicology and efficacy testing to occur, thus reducing the investment in each candidate until it is qualified. Secondly, as products proceed through development there is a need for grow-

ing quantities of the candidate material, from milligrams to tens of grams.

Also in parallel, quantities of low level impurities and metabolites to support compound identification and characterisation are needed. Typically this means continual redevelopment of a candidate's purification process as scale increases, because existing technologies cannot be directly and easily scaled. This in turn leads to further increases in cost and time during a candidate's development.

Therefore the pharmaceutical industry is beginning to focus on utilising process steps that scale, so that time and cost is not wasted redeveloping processes from analytical to process scale. Ideally such process steps should enable crude, unprepared reaction mixtures or fermentation broths to be processed.

Commonly over the last 10–15 years, solid phase chromatography plays a key and growing role, in the purification of candidates, via preparative HPLC or Flash chromatography techniques. However, as demands in the development process change, described above, solid phase technologies become increasingly expensive to use, both in time and cost and need other techniques to work along side them or in isolation to help achieve the goals of chemists.

The Liquid Alternative

One of the most interesting technologies being considered is a variant of partitioning chromatography, known as countercurrent chromatography (CCC). This technique uses a liquid, rather than solid, stationary phase to perform chromatographic quality purifications. Because we are now using a liquid for the stationary phase, you are able to access the entire volume rather than just the surface area, as is the case with solid phases. This allows greater loadings, total recovery of sample, no irreversible adsorption of candidate on to the solid phase, and new operating strategies to be used. Table 1 expands on these benefits.

Partitioning chromatography is a simple process to understand, since it is entirely dependent on the partition coefficient of a candidate between two immiscible solvents. Therefore it is very easy to directly scale from analytical through preparative to kilo scale and lends itself to the needs of the pharmaceutical industry.

Now that the stationary phase is liquid, it enables unprepared samples to be added directly to the column; obviously this is impractical with any solid phase. This also offers the further benefit of low pressure, again impractical with solid phases, which further supports the scalability of the technology.

Solving Purification Challenges

There are a number of key purification challenges that CCC is particularly suited:

1. Where solubility of your sample is problematic to your existing purification techniques.
2. Where your current separation strategies are proving ineffective in providing a solution.
3. Downstream processing of collected fractions.

The Liquid Alternative

The main benefits of using a liquid stationary phase for sample purification are:

Total sample recovery: As there is no solid phase, there is no adsorption onto a solid phase, so that a very high % of the target compound is recovered. Even if there is a problem, the sample can always be recovered, and the worst that can occur will be that the sample has been diluted.

Improved sample solubility: Since both the mobile and the stationary phase are liquid, the sample may be loaded in either one or the other, (or in both). Also there are many combinations of solvents that may be used, so that it is nearly always possible to find a combination of solvents which achieve the desired separation of any particular sample.

High throughput: In any real application, a key consideration is the volume of sample which can be treated per hour. High performance CCC, by its nature, enables higher loadings to be achieved.

Reproducibility and ease of scale-up: A liquid stationary phase can simply be changed by pumping out the columns contents and then pumping in fresh stationary phase. This means that the starting conditions are always the same, which in turn means that the level of reproducibility is extremely high. There are no unexpected surprises or degradations over time. Furthermore, because the separation is being achieved by mixing and settling two liquids, the underlying physical chemistry is the same at all scales, from mgs to kilo per injection.

Inherent flexibility: All separation techniques which employ a solid stationary phase can only be operated in elution mode. Having a liquid stationary phase means that the sample may be loaded in either the stationary or the mobile phase, and that there is a choice of hundreds or thousands of solvents. This inherent flexibility means that any particular separation may be optimised for any or several criteria, including resolution, time, throughput, reduce the solvent consumption, or cost.

Little or no sample preparation required: As both phases are liquids, and there are no capillary constrictions to cause blockages, samples may be loaded in crude form. This includes samples which may be viscous or which contain particles.

Table 1: Benefits of using a liquid stationary phase

Elution Strategies

Single-mode CCC: This is the standard method of elution. The stationary phase is retained while the mobile phase flows in one direction only. Either phase can be the mobile phase and therefore either normal (organic phase mobile) or reversed (aqueous phase mobile) phase separations are possible.

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 the 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 completely new and ready for the next injection.

Dual-mode: 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 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 elution strategy uses the phenomena that charged entities (ions) prefer the aqueous phases and uncharged molecules prefer organic phases. The strategy employs basic organic phases and acidic aqueous phases (or vice versa). The analytes dissolved in the stationary phase are eluted by the 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.

Co-current: Here the mobile and stationary phases are pumped simultaneously. Depending on the retained volume of each phase in the column, the residence time of each will be different, eluting the sample in a defined band whose volume or width is determined by the respective flows. This allows the stationary phase to be continuously refreshed, allowing continuous processing.

Table 2: Elution strategies available using a liquid stationary phase

	HPCCC	HSCCC	HPLC
Stationary phase	Lower phase of hexane-ethyl acetate-methanol-water (1:0.4:1:0.4, v/v)	Upper phase of light petroleum-ethyl acetate-tetrachloromethane-methanol-water (1:1:8:6:1, v/v)	Zorbax Eclipse XDB-C18 column 250 x 9.4 mm ID 5 µm
Mobile phase	Upper phase	Lower phase	Methanol-water (70:30, v/v)
Sample capacity per run g	43	2.0	1.96x10 ⁻²
Run time min	45	450	40
Productivity mg/min	431	4.44	0.49
Purity of isolated compounds	>99.9 %	>98.5 %	>99.0 %
Solvent consumption L/g	1.39	1.93	5.10

Table 3: Comparison of operating performance chromatographic techniques

The solubility of your sample in the mobile phase significantly impacts the throughput that can be achieved to produce a specified quantity with solid phase chromatography. CCC offers an alternative approach since both mobile and stationary phases are liquids, the process allows unpurified sample to be injected in either phase, without effecting the purification, thus expanding the options available to tackle the solubility issue.

In both solid and liquid stationary phase chromatography you perform a standard elution with only the mobile phase being pumped and obviously with solid stationary phase this is the only way that it can be operated. However, with CCC, using two liquids, other operating strategies are possible that either reduce the time and solvent consumption of the purification or enable the purification to be performed at high sample loadings. These alternatives are shown in table 2.

Finally it is often found with solid phase chromatography that there are problems with the processing of collected fractions. We typically find these solid phase chromatographic steps are performed in reverse phase, thereby generating aqueous fractions, which are laborious energy inefficient to provide the final compound and can lead to hydrolysis and degradation of the product. Additionally there can also be issues of eluting the entirety of the purified compound from the column. In CCC, the separations can be designed to be normal phase separations and obviously you do not suffer the elution problems highlighted earlier.

A Natural Example

The separation of two isomers Honokiol and Magnolol, which are the main bioactive constituents from Houpu, is a purification that is challenging, but necessary in the development of a Chinese traditional medicine. In the paper [1] by Lijuan Chen et al, they describe the method development, optimisation and scale-up using HPCCC equipment of two isomers.

In 2006, a paper by Lu, Sun and Pan [2] showed the relative performance of using HPLC

and HSCCC (High Speed Countercurrent Chromatography) to perform the same separation. In table 2 we have compared the results from these papers to demonstrate the purification power of the HPCCC equipment compared to HPLC equipment and other manufacturer's HSCCC equipment.

The comparison shows that HPCCC has the highest productivity by a factor of 100 while achieving the highest purity and lowest solvent consumption. This shows that HPCCC provides the least expensive isolation method for a pharmaceutical product such as Magnolol.

Conclusion

Pharmaceutical companies are looking for new technologies that help them achieve their goals of reducing the time and cost of their development process. HPCCC offers chemists and chromatographers working in this process a new alternative to solve their solubility and throughput requirements, while working with a technique that will not require redevelopment as scale increases.

References

- [1] Chen L., Zhang Q., Yang G., Fan L., Tang J., Garrard I., Ignatova S., Fisher D., Sutherland I.: The Rapid Purification and Scale-up of Honokiol and Magnolol using High Performance Countercurrent Chromatography (HPCCC), *J of Chromatogr. A*, in press
- [2] Lu Y., Sun C., Pan Y., *J. Sep. Sci.* 29, 351-357 (2006)

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