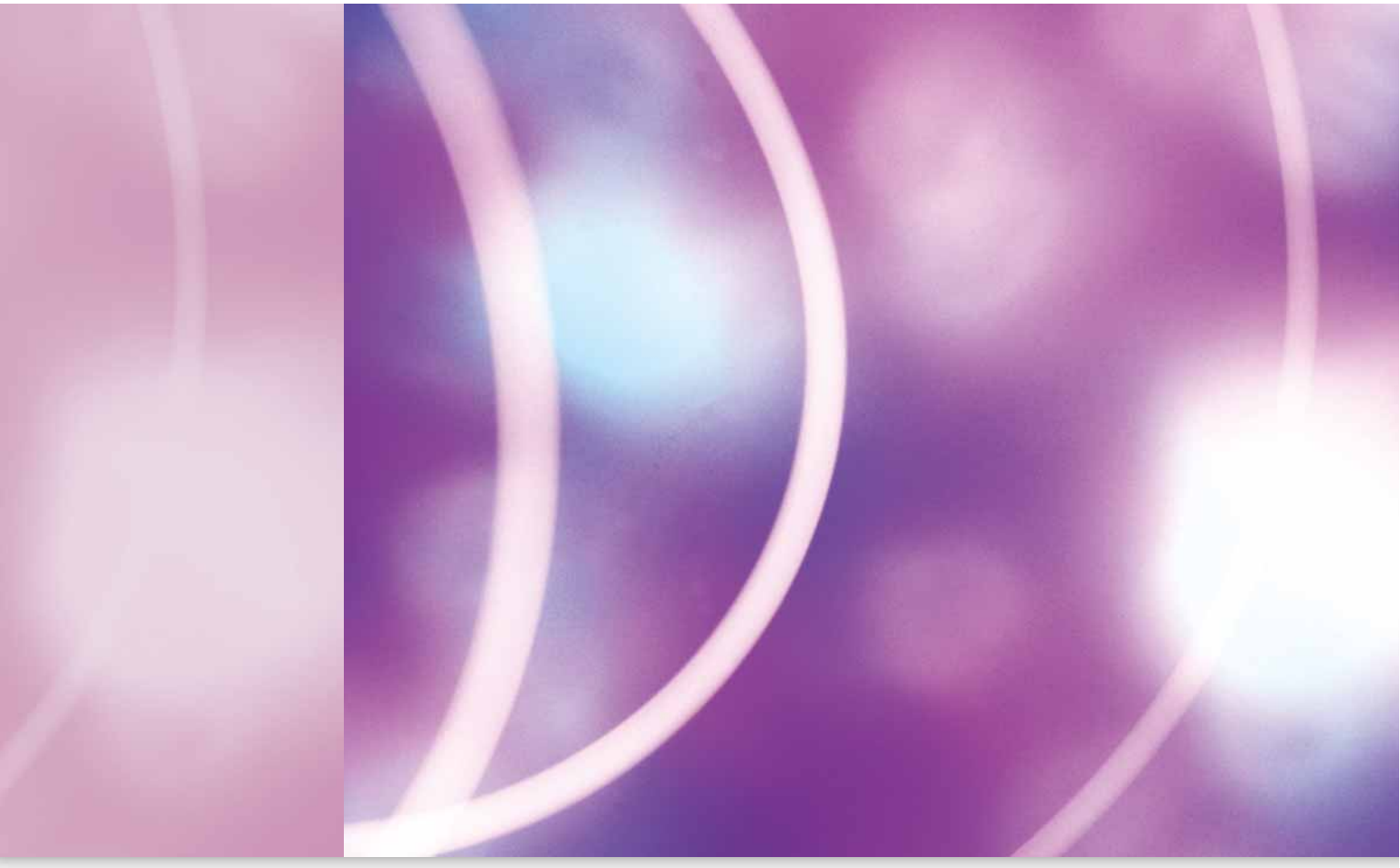

Advances in High Performance Countercurrent Chromatography

Dynamic extractions

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A look at the evolution of high performance countercurrent chromatography (HPCCC) as a purification tool.

Countercurrent chromatography (CCC) has been available to separation scientists since the early 1960s, but never gained the same popularity as analytical and preparative high performance liquid chromatography (HPLC), which are commonly used in the drug development process as a purification tool. CCC was largely ignored and viewed as a technique only to be used as a last resort when all other separation options have failed!

Most chemists appreciate the benefits of using two liquids for the mobile and stationary phases, including improved solubility capabilities, higher purification throughput and yields. So why has a technique based on partitioning chromatography become relegated to the bottom division of separation science?

This article explains why this has happened, what has changed and how chromatographers are benefiting from adding this tool to their purification armoury.

The Historical Limitations of CCC

There are three main reasons why CCC was not adopted when HPLC was being rapidly introduced:

Time of separation: Although "so called" high-speed CCC (HSCCC) machines were introduced in the early 1980s, they took many hours to perform a complete purification, whereas other liquid chromatography (LC) techniques performed similar tasks in minutes or tens of minutes.

Range of equipment available: Previously the only size of HSCCC machine available was at the semi-preparative scale (i.e., high hundreds of milligrams to small grams). At the early stages of a drug development programme, this size of injection might be the total amount of material available and would never be risked as a single injection.

Further down the drug development process there is a requirement to process tens to hundreds of grams of material. Because there were no larger HSCCC machines available, purifications using this technique were impractical.

Reliability of equipment: Until the late 1990s the reliability of HSCCC instruments was questionable. Given the value of the products requiring purification, it is hardly surprising they were not risked in these instruments.

The Evolution of CCC

Research funded by UK research bodies began in 1996 to overcome the drawbacks associated with CCC and significant advances in CCC equipment as a purification tool have been made (Table 1).

The investigation began by examining and experimenting with the existing HSCCC equipment. This highlighted the engineering challenges needed to be overcome to achieve equipment with high stationary phase retention which require equipment of a far higher g-level. This led to the strategy of

developing machines that could generate far higher g-levels than had previously been possible.

HSCCC machines could generate around 80 g, whereas a HPCCC version would generate 240 g. A prototype was subsequently built which showed that high stationary phase retention and a high purity chromatographic separation could be performed in 15–30 mins, rather than the hours that HSCCC takes. This prototype also demonstrated that analytical-scale CCC was possible and became the first of this type of machine available.

The increase in g-level that could be obtained was a fundamental breakthrough in the engineering design of HPCCC equipment. This advance coincided with the conclusions of research into the use of increasing bore sizes to provide large columns. This work revealed that the use of larger bore columns was practical and was a sensible engineering route to design larger-scale CCC equipment for purification purposes.

This new knowledge was quickly incorporated into the engineering design of a preparative-scale HPCCC unit to demonstrate the improvement in performance. New designs were then conceived for pilot/kilogram-scale equipment. The first prototype pilot-scale HPCCC unit was commissioned in 2004 and demonstrated the purification performance predicted. This showed that purification at a milligram to kilogram scale was now available for chemists and chromatographers. One further result of this work showed it was possible to volumetrically scale from an analytical separation directly to gram- and kilogram-scale injections.

HPCCC

High performance countercurrent chromatography equipment gives the chemist and chromatographer an additional tool to solve their current purification challenges. The use of HPCCC equipment has been found particularly effective where scale-up or sample solubility or crude sample condition are causing purification problems, such as the ability to perform the separation in the first place through to the current method being uneconomic or time consuming. The use of HPCCC equipment is particularly effective where scale-up, sample solubility or crude sample conditions are causing purification problems in terms of separation performance, expense, or time consumption. HPCCC offers various strategies to overcome problematic purification steps (Table 2).

Purification using HPCCC equipment is a result of partitioning. Both mobile and stationary phases are immiscible liquids. This allows a liquid stationary phase to be used and a broad range of molecules can be purified.

In solid-phase chromatography the solubility of the sample in the mobile phase significantly influences the throughput that can be achieved to produce a specified quantity. HPCCC offers an alternative approach since both mobile and

Table 1: Key dates in the development of CCC equipment.

1999: Project established to improve upon HSCCC capable of processing 1 g of crude sample an hour.[1] The relationship between mobile phase flow rate and stationary phase retention was established.[2]

2000: Stationary phase retention is improved by using a "J" type centrifuge.[3]

2002: A 5 mL instrument capable of performing model separations in minutes, demonstrates the possibilities for HPCCC.[4]

2003: A DTI SMART research award allows construction of prototype preparative HPCCC equipment.

2004: A 4.6 L HPCCC machine was developed.[5]

2005: Technology transfer of a glucoraphanin extraction, demonstrates HPCCC performance at a scale previously unreported.[6]

2006: Volumetric scale-up demonstrated.[7] 1 L HPCCC instrument used to process 37 g of crude sample per hour highlights the effectiveness of this technique.

Table 2: Modes of HPCCC.

Elution extrusion: This strategy takes advantage of the fact that the analyte may be fully separated inside the column before being eluted. Because a liquid stationary phase is used, it is possible to recover the separated compound without completing a full elution cycle.

In elution extrusion, the separation begins in the same way as single-mode CCC. However, when the run reaches a certain point the mobile phase is stopped and the stationary phase is pumped in to extrude the column contents. This enables the purification cycle and the amount of solvent used to be significantly reduced. After extrusion, the column is completely replenished and ready for the next sample injection.

Dual-mode elution: When operating a dual-mode elution strategy, the aqueous phase is initially pumped as the mobile phase (i.e., normal phase operation) and after a set period of time the organic phase is pumped as the mobile phase (i.e., reverse phase operation). This switching procedure is repeated until the desired resolution for purification is achieved.

The advantage of this method is that compounds that have a 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 utilizes the phenomena that charged entities (ions) prefer the aqueous phases and uncharged molecules prefer organic phases so uses 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. This strategy enables a very high loading capacity to be achieved with high resolution separations for those molecules that have the necessary characteristics.

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.

Another drawback of solid-phase chromatography is that you can only perform a standard elution by pumping the mobile phase. However, with HPCCC either liquid phase can be pumped permitting other operating strategies to be explored, to reduce either the time or solvent consumption of the purification or enable the purification to be performed at high sample loadings.[1]

Finally, with solid-phase chromatography there are problems processing the collected fractions. Solid-phase chromatographic steps are typically performed in reverse phase, which generates aqueous fractions that are laboriously 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. Problems can also arise when using a solid stationary phase as there is always a possibility of irreversible adsorption of the sample onto the solid phase. The entire sample cannot, therefore, be retrieved. This problem is eliminated with HPCCC because two liquids are used.

In CCC, the separations can be designed to be normal phase separations and you do not suffer the elution problems highlighted earlier.

Figure 1: Scaling up from analytical to semi-preparative levels with minor impurity removal.

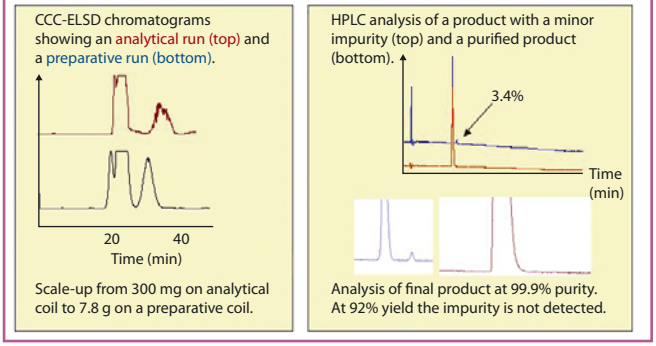
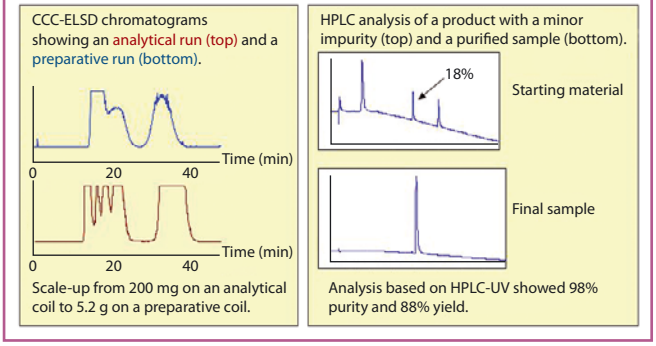


Figure 2: Scaling up from analytical to semi-preparative levels with impurity isolation for identification purposes.



Working with a liquid stationary phase allows a greater range of solutions to solubility problems that are encountered and once a separation is developed, it can then be scaled seamlessly from milligram to kilogram levels — and beyond. This ensures that chemists and chromatographers spend their time developing drugs not chromatography methods.

Two examples of separations performed using HPCCC equipment are shown in Figures 1–2. Both separations show the technique directly scales and each was developed and the target compounds isolated all within one working day. In these particular examples, the work was done at the required scale in a single day because of the loading that could be achieved using HPCCC equipment. If another technique, say HPLC had been used, assuming it was possible to perform the separation, then the loading per injection would have been far less and more time would have been taken to perform the same work.

Conclusions

This article describes how CCC equipment has developed over the last 10 years and how HPCCC equipment can be used as a purification tool for the pharmaceutical industry. This additional purification approach provides new alternatives. Areas where HPCCC is currently being performed are in the purification of molecules from natural sources so they can be studied again in the discovery and development of new drug products.

Acknowledgement

S.T.A. Dubant, of Pfizer, for producing the chromatograms in Figures 1 and 2.

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The Column

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