High Performance Countercurrent Chromatography (HPCCC) finally allows the advantages of liquid/liquid chromatography to be used in mainstream purification in medicinal chemistry

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Chemists have known of the potential benefits that liquid/liquid chromatography would have over solid/liquid chromatography for many years. Yet solid/liquid chromatography techniques, such as HPLC or Flash have become the workhorses of purification, while, until only recently liquid/liquid chromatography, namely Countercurrent chromatography (CCC) has been barely used and then primarily a technique for natural products or academic research.

The lack of uptake happened due to the limitations of early CCC instruments that were often poorly engineered and unreliable. Purifications took hours and due to analytical scale instruments not being available the minimum sample required was in the region of a gram. It is, therefore easy to understand why the status quo developed.

High Performance Countercurrent chromatography is the rebirth of liquid/liquid chromatography in the 21st century

However, a new generation of CCC instruments, High Performance Countercurrent Chromatography (HPCCC) instruments, has led to the rebirth of liquid/liquid chromatography in the 21st century. These instruments range from analytical scale through to kilo, while performance has been significantly enhanced reducing purification times to minutes. The development of HPCCC instruments combined with the limitations that chemists are experiencing with solid/liquid chromatography techniques, has created an environment where chemists are using the benefits that liquid/liquid chromatography can offer them to solve current purification challenges.

Because HPCCC is a high capacity technique, it is becoming the first choice for scientists when they need to produce large quantities of target compounds (i.e. high purity product for

studies in phase 1, 2 or 3 clinical trials or for impurities, normally found in low concentration, that need to be produced in quantity for standards).

This is especially attractive when a library of compounds, are identified as a lead candidate that will need to be produced in ever increasing quantities, as they progress through the pharmaceutical development process.

Using HPCCC instruments chemists are able to concentrate on their product development process not purification/chromatography redevelopment, as scale increases.

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Performing scale-up of a purification between differing capacity HPCCC instruments is quick and simple. HPCCC instruments create the same operating conditions and you simply use the volumetric ratio between the two column volumes you wish to use to determine the new sample volume and mobile phase flowrate.

A further significant benefit concerns sample solubility. Often a limiting factor with HPLC purifications, this is all but eliminated. With HPCCC instruments your sample can be injected onto the column in either mobile, stationary or a mixture of both phases, without affecting the performance of the

chromatography. This is shown in the two pictures below:

Put simply solubility is far less of an issue when working with liquid/liquid chromatography, and is not a limitation on the throughput you need to achieve. To demonstrate this we have shown below the sample for the high purity purification of glucosinolates, where the loading achieve is 50% weight/weight and shows how samples containing solids can be loaded, without affecting operation or performance of the HPCCC instrument.

Making liquid/liquid chromatography accessible – The development of HPCCC instruments

Liquid/liquid chromatography has long been inaccessible to chemists due to unreliable instrumentation with poor purification performance and with no range of instruments able to scale between milligram to kilo and beyond.

The breakthrough occurred when these HPCCC instruments were engineered to run at 240g instead of running at g-levels of between 60g and 80g. These low g-level machines were collectively known as high speed countercurrent chromatograms (hsccc). This breakthrough delivered two significant aspects of instrument performance:

1. Firstly, separation time was reduced by a factor of 10, so now these high capacity separations could be performed in times similar to solid phase chromatography, such as HPLC or Flash.
2. Secondly, it makes analytical scale purifications practical and quantifiable, by allowing small-bore tubing to be used without loss of resolution.

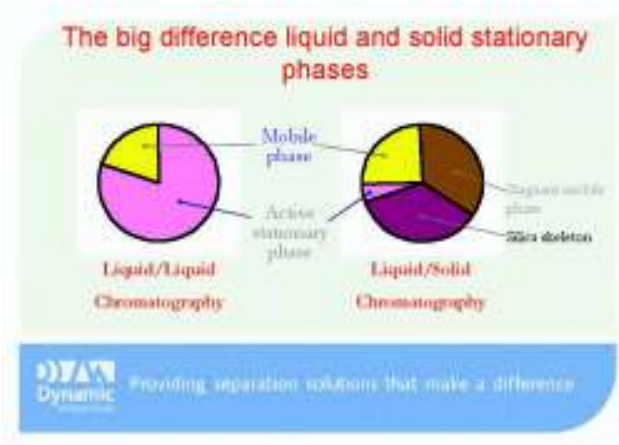
At the same time, all the old hsccc equipment design faults that worried scientists have been engineered out of the new HPCCC design. No more bolting machines to the bench, having to rewind columns after a few runs, flying leads failures during the first run or working in the back room because colleagues cannot stand the noise of the unit. HPCCC instruments are quiet, reliable and robust allowing them to continue to perform constantly to high quality..

With these improvements and developments scientists can now chose from a range of instruments to match their requirements for purification in a pharmaceutical environment. HPCCC instruments can simply be considered as an addition to the scientist's chromatography armoury and are simply installed, as shown below.

The power of using liquid stationary phases

The key benefits of using a liquid, rather than a solid stationary phase can be summarised as follows:

- High injection loadings
- Improved sample solubility
- Ease of scale-up
- New elution strategies
- Total sample recovery
- Little or no sample preparation



However, for the purpose of this article we are solely going to focus on high injection loadings. Firstly, you have to understand the difference in the amount of accessible stationary phase present when one compares liquid/liquid to a liquid/solid chromatography. It is schematically shown in the diagram above, but there is a 10 to 20 times difference between the two techniques. Liquid/liquid chromatography is volume chromatography compared to liquid/solid chromatography taking place on a surface.

The power of CCC is the selectivity of the solvent systems

However, having so much capacity is of little worth if one cannot resolve your target compound. Scientists very quickly recognise that a technique based solely on partitioning will only have a small number of theoretical plates and therefore might consider the technique of limited use.

This is a misconception. The inherent power of this technique is given to it by the selectivity of the solvent system. CCC is a technique that is very good at separating target compounds so that their peaks are widely spaced. This is why the technique can achieve high sample loadings, dramatically improving chromatography throughput. This is demonstrated in the example shown below, where despite only a minimal change in the number of theoretical plates the resolution of the components have been dramatically improved by changing the solvent system.

Showing the selectivity of the solvent systems

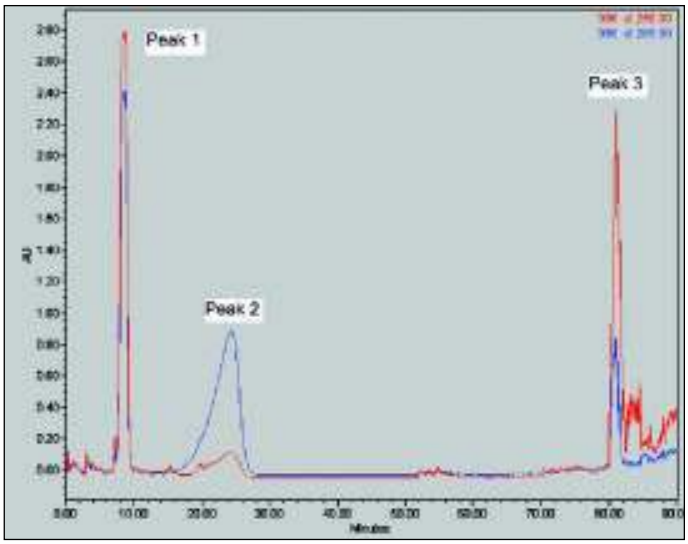
An example will demonstrate the selectivity of the solvent systems. A UK pharmaceutical company suggested a mixture of synthetic

No	Heptane	EtOAc	MeOH	Butanol	Water
1		0	0	2	2
2	0	0.4	0	1.6	2
3	0	0.8	0	1.2	2
4	0	1.2	0	0.8	2
5	0	1.6	0	0.4	2
6	0	2	0	0	2
7	0.1	1.9	0.1	0	1.9
8	0.2	1.8	0.2	0	1.8
9	0.29	1.71	0.29	0	1.71
10	0.33	1.67	0.33	0	1.67
11	0.4	1.6	0.4	0	1.6
12	0.5	1.5	0.5	0	1.5
13	0.57	1.43	0.57	0	1.43
14	0.67	1.33	0.67	0	1.33
15	0.8	1.2	0.8	0	1.2
16	0.91	1.09	0.91	0	1.09
17	1	1	1	0	1
18	1.09	0.91	1.09	0	0.91
19	1.2	0.8	1.2	0	0.8
20	1.33	0.67	1.33	0	0.67
21	1.43	0.57	1.43	0	0.57
22	1.5	0.5	1.5	0	0.5
23	1.6	0.4	1.6	0	0.4
24	1.67	0.33	1.67	0	0.33
25	1.71	0.29	1.71	0	0.29
26	1.8	0.2	1.8	0	0.2
27	1.9	0.1	1.9	0	0.1
28	2	0	2	0	0

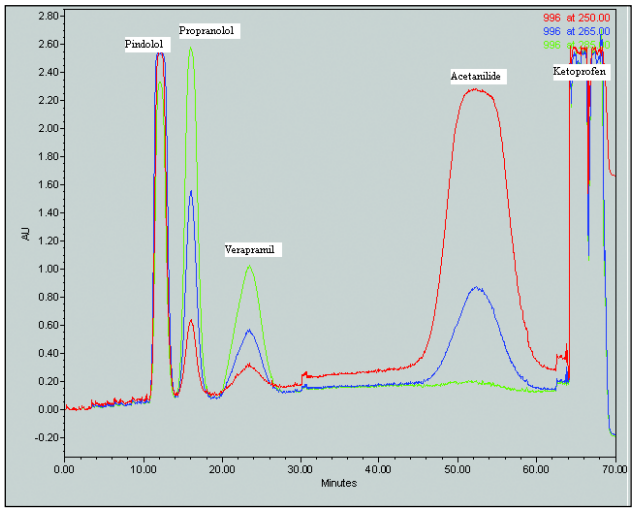
HEMWat solvent system table

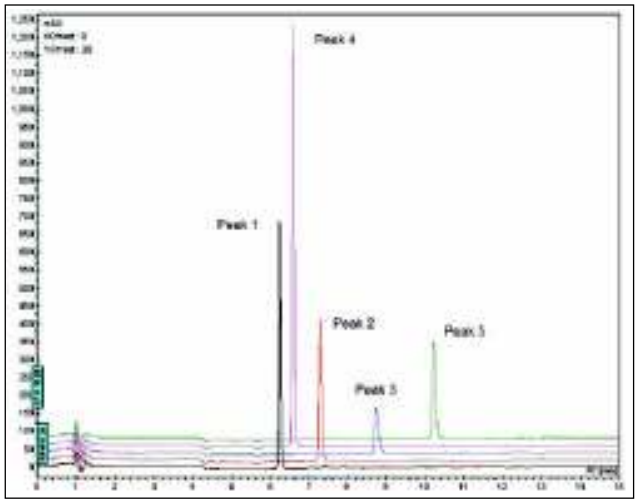
standards to be used during their proof of concept study of HPCCC instruments. The mixture has five components of mixed polarity (Pindolol, Propranolol, Acetanilide, Verapamil and Ketoprofen). To demonstrate how peaks can be moved we first selected solvent system 6 from the table below:

Once the column was equilibrated, a sample of 1 milligram of each standard (5 mgs in total) was then injected onto the column, and the following chromatogram obtained:



What can be seen is that after 60 minutes only 3 peaks have been eluted and detected, whilst the other two compounds are well retained in the stationary phase. However the sample was then separated using solvent





system 11. Note how the peaks have been moved, against the time axis, in the chromatogram that follows.

This illustrates how simple changes in the solvent system can reshape the separation, allowing all five compounds to be separated with baseline resolution as shown by the following HPLC analysis of the peaks collected:

Extra chromatography capacity in your laboratory

So what does this all mean for chemists and separation scientists? The ability to use liquid/liquid chromatography in the laboratory brings high capacity separation instruments to the bench top. It is now possible to utilise pumps working at 50 ml/min process 200 to 400 grams of crude material per day.

This is a significant advance in reducing the chromatography bottleneck that exists

This is a technology advance that can significantly reduce the chromatography bottleneck. Whether caused by throughput constraints of your liquid solid chromatography techniques or the solubility of your samples, HPCCC instruments can help you solve these issues.