Preparative Chiral Separations – from Laboratory Scale to Production

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Over the past few years, preparative chromatographic separation of racemic mixtures into their individual enantiomers has become an integral part of the development process for new drug entities. This is because the number of chiral drug candidates has been increasing, a not surprising development, given the asymmetric nature of the drug receptor sites. At an early stage in development it is essential to know the differences in activity and toxicity between the two enantiomers in order to maximize the effectiveness of the product while minimizing the possible negative side effects of the new drug. At this stage of the process there is practically nothing known about the chemistry and physical properties of the molecule and the fastest and most convenient way to the pure enantiomers is usually chromatographic purification. In contrast to other possible procedures, only a few mg of product and a few hours are needed to develop a chromatographic separation method – important for these new candidates where there may be only a few hundred mg of the product in the world.

Once developed, the chromatographic procedure can be used both to analyse the optical purity of the product and to isolate the enantiomers. In the drug development process, speed to market is vital and rapidly reaching a go / no-go decision point for the development is critical in resource allocation. Thus, as the new drug moves through the early stages of development and increasing amounts of material are needed, the initial chromatographic method can grow in scale with the needs of the project, often reaching the isolation (under cGMP) of kilogram quantities for Phase 1 clinical trials. At this point the immediate pressure of development eases, allowing a more leisurely investigation of the possible processes to make the desired enantiomer. The focus at this point is to find the most economical procedure for the production of the material in time for Phase III, where the manufacturing process is typically locked in and all alternative processes from crystallization through asymmetric synthesis are investigated. In some cases, chromatography remains the option of choice while in others the alternative procedures are chosen. While the aim is generally to use the process that results in the lowest cost per kg of the final product, the choice may also be influenced by capital expenditure requirements or by concerns about the scalability of the process. While the latter concerns should by now be alleviated by the success of the current production scale chromatographic enantiomer separations, the capital expense of installing a large scale chromatographic system as opposed to utilization of existing tankage (for a crystallization, for example) could result in a decision to use a more expensive but less capital intensive process.

Considerations of scale.

In the progression from the small scale chromatographic purification to production scale operations there are many changes made in both the chromatographic methodology and its philosophy. At the smallest scale, cost is not important and the need is to find an adequate separation method in the shortest possible time which can produce the few tens to hundreds of mg. At this scale the separation time for the isolation is short; there is little purpose in spending several days to develop an optimised separation. As the scale increases, there is increasing emphasis on the economics of the separation. Despite the high overall costs of bringing a new pharmaceutical product to market, the costs of individual steps remain under strict scrutiny and the chromatographic method frequently is optimized and in some cases may be redeveloped in order to meet the cost requirements. Much more care is taken to find a high selectivity and to optimize the separation when the scale increases to the few hundred grams needed for toxicology or the kg quantities for Phase 1 trials. The transition to large scale processing beyond Phase 1 is usually accompanied by a transition from conventional batch chromatographic separation techniques to the production-scale oriented simulated moving bed technology. This continuous chromatographic process is generally more cost effective than conventional single column chromatography, combining use of significantly less solvent and stationary phase with higher productivity, but it requires more optimization and development time than the simpler batch process.

Early Stages.

Separation method development time has to be short in the early stages of the development of the new product to meet the stringent time constraints. Methods are typically developed by screening a small set of enantioselective columns with the aim of finding a baseline separation quickly. Increasingly (in the USA at least) this is done using supercritical fluid chromatography; replacement of organic solvents with a mobile phase predominantly consisting of supercritical carbon dioxide results in approximately a fourfold reduction in solvent viscosity. This allows the columns to be operated at four times the flow velocity used in corresponding HPLC methods, dramatically reducing the screening and separation time. SFC methods also result in the use of smaller volumes of organic solvent during the separation process. While efficient solvent recycling procedures minimize the environmental impact of this reduction in solvent use relative to HPLC, the products are isolated in smaller volumes (often 5 to 10 times less) than in HPLC. This reduces the evaporation time and results in a little less energy use in the process (though it should be noted that operation in SFC involves several phase transitions which consume more energy than simply pumping solvent as in an HPLC system). Although SFC is widely used at this stage of development, this does not mean that HPLC processing should be avoided or

ignored. There may be advantages in an HPLC process – better selectivity or solubility, for example – which allow faster purification despite the lower flow rates typically used. Sometimes separations can be achieved using one of the techniques and not the other; thus it is worth screening both, especially where the scale of separation may be increased at some time in the near future and the most effective separation will be required.

Method Development.

Whether one is developing an HPLC or an SFC separation, the procedure is very similar. As there is currently no way to predict which column – mobile phase combination will give a separation of the desired product (and it is probable that such a prediction will continue to elude scientists in this field for some years to come!) the method development process generally involves screening a number of chiral stationary phases and potential mobile phases in a systematic scheme. This is aided by statistical information which tells us that for past separations there are sets of chiral phases which will give at least an 85 to 95% chance that such a set will provide conditions suitable for the preparative separation. This is not, of course, a guarantee, especially when new molecular structures are in development. Typical sets of columns and mobile phases for primary HPLC screening are shown in Table 1. If this initial screening is not successful, typically one moves to a secondary screen, where the lesser used columns and solvents are employed, again in a similar process. Usually the column sets are mounted on switching valves in the chromatograph and the whole is operated automatically, allowing much of the screening process to be run

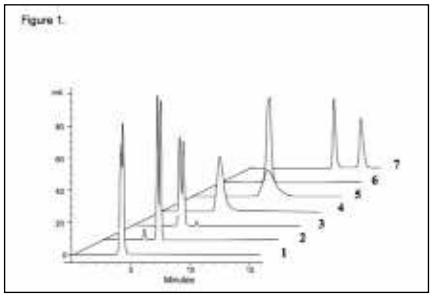

Figure 1. Screening results for benzoin ethyl ether. Columns 250 x 4.6 mm. Mobile phase hexane : 2-propanol (85:15), flow rate 1 ml/min. Columns: 1: CHIRALCEL OD; 2: CHIRALPAK AD; 3: CHIRALPAK AS; 4: CHIRALCEL OF; 5: CHIRALCEL OB; 6: CHIRALCEL OG; 7: CHIRALCEL OJ.

overnight in an unattended fashion. A typical screening result is shown in Figure 1. For larger scale separations it is often most convenient to run a full screen of all available columns and mobile phases for the separation since at this point the best rather than a merely adequate separation is often required. Screening in this case can be an involved process. At Chiral Technologies, for example, a full screen involves more than 100 solvent – column combinations while a screen for an industrial process in which at least 70 to 80 additional chiral phases are investigated involves even more. Such a full screen can take a long time to complete and ways to reduce this are continuously researched. Besides the use of SFC, which as noted above reduces the analysis time by a factor of around 4 from HPLC, screening can be accelerated by use of smaller particle size columns. A column 5 cm in length packed with 3 micron particles will have higher efficiency than the 15 cm column packed with the 20 micron CSP often used for larger scale separations and can give selectivity and retention data in an order of magnitude less time. It is essential, of course, that the small particles have chromatographic properties identical with the larger particles that will be used for the separation project. Parallel chromatography systems have been developed as another approach to rapid screening. These typically use 8 channels with either conventional columns (Sepiatech, both HPLC and SFC) or microflow columns of 0.3 mm id (Eksigent). Such parallel systems allow a screen of 8 columns in the same time as conventionally used in screening just one. Coupled with solvent switching to allow fully automated screening gives these systems an 8-fold time advantage over the conventional single channel units.

(a) Immobilised polysaccharide-based phases

Columns+:	1. CHIRALPAK® IATM (immobilized amylose tris(3,5-dimethylphenylcarbamate))
	2. CHIRALPAK IBTM (immobilized cellulose tris(3,5-dimethylphenylcarbamate))
	3. CHIRALPAK ICTM (immobilized cellulose tris(3,5-dichlorophenylcarbamate))
	+ Other solvent-stable chiral columns such as Whelk-O 1 (etc) may be included in the set.
Mobile phases:	1. Hexane – 2-Propanol (80:20)
	2 Hexane – Ethanol (80:20)
	3. Methyl tert-Butyl Ether – Methanol (98:2)
	4.Hexane – Dichloromethane – Methanol* (49:49:2)
	** Alternatively Hexane – THF – methanol may be used in place of the chlorinated solvent.*

(b) Coated Polysaccharide-based Phases.

Columns:	1. CHIRALPAK AD® (amylose tris(3,5-dimethylphenylcarbamate))
	2.CHIRALCEL® OD® (cellulose tris(3,5-dimethylphenylcarbamate))
	3.CHIRALPAK AS® (amylose tris(S-α-methylbenzylcarbamate))
	4.CHIRALCEL OJ® (cellulose tris(4-methylbenzoate))
Mobile Phases	1. Hexane – 2-Propanol (85:15)
	2.Hexane – Ethanol (80:20)
	3.Methanol (100%)
	4.Acetonitrile (100%)

The solvent strength of the mobile phases used in screening should be adjusted to obtain reasonable elution times by changing the proportion of the polar (alcohol) modifier.

(CHIRALPAK, CHIRALCEL, AD, OD, OJ and AS are registered trademarks of Daicel Chemical Industries, Ltd.)

Table 1. Screening conditions for HPLC Method Development

Optimisation.

Once screening is complete, the separation is generally optimized to maximize the selectivity and to bring retention times into an acceptable window. This process can be more time consuming than the screening, especially as this step relies on the expertise of the chromatographer to develop the most effective procedure. For HPLC processes, it has been calculated that the optimum retention factor for the first peak in the chromatogram should have a value around 1[1] . For SMB processes (see below) this value should be reduced for maximum production rate [2]. Optimisation also may include investigation of the sample solubility; if a solubility of only a few g/l is attained, the preparative method will always be slow and expensive. In this respect, the use of a combination of immobilized chiral phases and mid-polarity range solvents such as dichloromethane, ethyl acetate and THF (see Table 1) have been found to be extremely useful; many drug candidates are not especially soluble in the more conventional hexane – alcohol mobile phases employed in chiral chromatography . The method development process is completed by a loading study in which increasing quantities of the racemic compound are injected to the point where the two enantiomer peaks overlap. For small scale separations this process is stopped at the point at which the two chromatographic bands just touch. As the scale increases it may be better to sacrifice some recovery in favour of increasing the production rate of the separation by increasing load further, allowing the bands to overlap and taking the appropriate fractions which give the desired combination of purity and product yield.

Particle size and column technology.

At this point it is also necessary to make decisions on the particle size of the media that will be employed in the larger scale separations. Small particles, while they give high separation efficiency and allow difficult separations, produce high operating pressures. This is not an issue in small scale operations (up to ~ 5 cm id columns) for many

reasons. A major factor is one of time. In the transition from small to large particles there is a finite redevelopment time where the separation is modified to account for the lower column efficiency. Where the selectivity is high, this is not important, but for the more difficult separations there can be a significant loss in production rate. As most HPLC and SFC systems can cope with the pressures required to run semi-preparative columns at a reasonable flow rate the simplest and fastest option is to use the same particle size for the preparative separation as for the analytical scale column used for development. For larger scale separations the particle size becomes important as the column diameter is necessarily increased. For columns 10 cm id and above it is necessary to limit the operating pressure to prevent damage to the silica base particles since wall support for the chromatographic bed is lost in such wide diameter columns. Just as importantly, the production rate needs to be maximized for these larger scale separations to minimize the project duration and the costs. Larger particles of 10 to 20 microns diameter allow higher flow rates (albeit at a loss in plate count, which for the higher selectivity separations is less important a parameter) which give higher production rates. Thus larger particle sizes are preferred as the scale of operation increases, with SMB processes optimally operating toward the 20 micron end of the range.

The column technologies available for preparative chromatography have changed little over recent years. Axial compression technology, introduced in the 1980s[4], revolutionized the preparative technique by allowing stable, high performance columns of diameters greater than 5 cm to be prepared from the small particles used in HPLC separations. Several variations on this theme have appeared more recently, but all such columns perform similarly with the compression technique compensating for the inevitable voiding and channeling that plagues large diameter columns. For columns 5 cm and less, there are several techniques used to pack high performance columns, some relying on axial compression schemes, others using more traditional high pressure slurry processes. For these, the performance of columns packed by different technologies is closely similar; a well-packed column has the same performance characteristics and lifetime regardless of how it is prepared.

Supercritical Fluid Chromatography (SFC).
As noted above, SFC has supplanted HPLC as a preparative technique in many companies which are concerned with small scale separations at the discovery level. The reasons usually cited for this change in processing are the faster separations, due to the low mobile phase viscosity, and the reduction in organic solvent consumption which results in easier product recovery. The technique is promoted as being "green" in that it uses less solvent (the carbon dioxide used in the systems is usually a by-product of other processes; its use in SFC separations merely delays its arrival in the atmosphere) and as such can make a small difference to the overall carbon emissions from the industry. Although SFC saves costs in terms of the low price of CO2, it must be remembered that it is more expensive to operate, as the pumps required for the CO2 are considerably larger than those required for similar flow rates of organic solvents and there are several phase changes through the cycle (see below) which require energy input. Unlike the situation for HPLC, the mobile phase in SFC is a compressible fluid at high pressure which requires significant safety considerations to be taken into account in equipment design and operation.

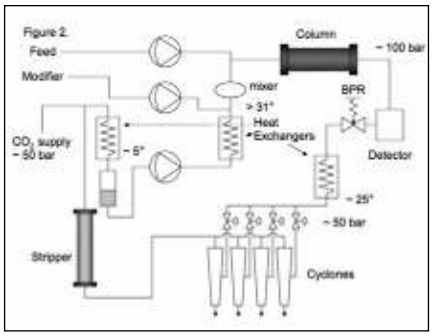

Figure 2. Schematic of a Preparative SFC unit.

A schematic of a preparative supercritical fluid chromatographic system is shown in Figure 2. The key differences from HPLC systems lie in the use of carbon dioxide as the main component of the mobile phase. CO_2 is non polar and for almost all applications a mobile phase modifier has to be used to increase the overall solvent polarity to solubilise the sample and to allow elution from the column. The CO_2 has to be in the supercritical fluid state (or close to it) for the chromatographic step which means it has to be pressurized to greater than 73 bar at a temperature of greater than 31.1°C. In order to bring it to the required pressure it has to pumped, which means it needs to be in a liquid form at this point. This is usually accomplished either by using a cylinder with a dip tube or by condensing gaseous CO_2 by maintaining the pressure at around 50 bar and reducing the temperature to a few degrees above 0°C. Once the operating pressure is reached, the temperature is raised to bring the CO_2 to the supercritical state after which it is mixed with the mobile phase modifier. The sample, dissolved in the modifier, is introduced from a separate pump or from a loop injector. After the separation and the components are detected, the pressure is reduced in the back pressure regulator (BPR) to bring the supercritical fluid to the gaseous state. This pressure reduction results in rapid cooling and the temperature has to be controlled to prevent the equipment from being encased in a block of ice. Once the CO_2 is a gas, the solubility of both the samples and the mobile phase modifier becomes extremely small and these components drop out of solution as a fine mist. Collection of the organic components is usually done in a cyclone collector which efficiently separates out the mist, condensing the product as a solution in the mobile phase modifier. The carbon dioxide is then either vented to the atmosphere or is recycled back to the pump through a stripper to remove remnants of modifier or solutes. In the latter case, the pressure downstream from the backpressure regulator is maintained at around 50 bar and the gaseous CO_2 is condensed by cooling the stream.

One aspect of SFC that is currently problematic lies in sample introduction. The sample is usually introduced into the mobile phase stream with a loop injector or a sample pump as a solution in the organic modifier. This results in band distortion when the sample volume is large because the pulse of strong solvent causes premature elution of the solute molecules within it as it mixes with the mobile phase. This distortion can limit the injection volume that can be used. An alternative, to introduce the sample into the modifier stream before mixing with the CO_2, results in broader injection bands, especially when the modifier concentration is low. Another problem that can arise is that of sample solubility. A not infrequent situation is where the sample, or a sample component, is less soluble in the supercritical mobile phase than it is in the modifier. As the mobile phase and injected sample mix, the sample – or the insoluble component – may precipitate prior to reaching the column inlet. This often results in pressure increases on injection and can result in blocked and distorted frits, which destroys the column (Figure 3). The ideal solution, to dissolve the sample in the supercritical mobile phase, is not easily implemented and is not offered in commercial systems.

Figure 3. Result of Inlet Frit Blockage and Consequent Over-pressure in an SFC Column. CHIRALPAK AD-H, 250 x 50 mm.

High Performance Liquid Chromatography.
HPLC has been around for many years and although at the small scale end of preparative chromatography it is being supplanted by SFC, nevertheless it remains the more important technique at larger scale. This is partly due to the size, availability and cost of large scale SFC equipment, as well as the services and costs required to run it. In labs at

Chiral Technologies we screen both HPLC and SFC screens are conducted, choosing the technique which gives the most economical solution, although HPLC becomes the preferred methodology for projects in excess of around 1 kg. As the simpler process, HPLC retains several advantages over SFC, in that sample cannot be lost during fraction collection, safety precautions are less stringent as the mobile phase is an incompressible liquid and as there are no phase changes, heating and cooling services are not necessary.

Separations in HPLC are usually scaled to "touching band" level, where the sample load is increased to the point where the front of the second band starts where the tail of the first eluted component reaches the baseline. Displacement effects are not as strong in chiral separations as in many achiral situations for a number of reasons and recovery of valuable material is often a priority so heavier loading is rarely used.

Simulated Moving Bed Chromatography (SMB).

SMB as a process for the pharmaceutical industry was implemented in the mid 1990s as an adaptation of the large scale processes for *p-xylene* and high fructose corn syrup. SMB is a multi-column, countercurrent continuous binary separation process and is preferred on the basis of process economics as the scale of the separation increases toward production . There are currently several enantiomerically pure pharmaceutical products that are produced at a manufacturing scale (ie multi-MTA) using this technique.

Although at first sight it appears to be complex, it is based on simple chromatographic concepts. As bands separate in a column they move at differing speeds. If we could move the stationary phase as well as the mobile phase, then moving it in the opposite direction at a speed intermediate between the two band speeds would result in the slower moving band being transported with the stationary phase while the faster one would move with the mobile phase. If nothing else happens, the two bands would move further apart with time, leaving an unused space in the centre of the column. This means that one can introduce the feed continuously into the centre of the column and the two components would continue to separate. The products are removed by bleeding off material from the pure zones at the outer ends of the band. As the stationary phase cannot be moved while maintaining a well-packed bed, the entire column must move. This is accomplished by using multiple columns in series, with movement affected not by moving the columns but by moving the inlet and outlet positions instead.

Unlike the situation in HPLC, where it is straightforward to design the preparative separation from a series of mass-overloaded injections, SMB requires a more complex procedure; usually computer simulations are used to develop operating conditions suitable for the separation followed by experiment to "fine-tune" the conditions thus developed. The data from the HPLC loading study is used to determine the parameters for the adsorption isotherms of the components which are then used in the computer simulations. Empirical determination of the operating conditions, although it is somewhat slower, is fortunately not too exacting a task and is normally used for the situations where the adsorption isotherms are not well described by a theoretical model.

An excellent account of the development of a large scale manufacturing process by SMB has been written and although it is not the purpose here to go deeply into a description of such a procedure, there are some basic principles that can be noted. At the laboratory scale, the most precious resources are time and manpower. Thus, separations are generally designed to take the shortest possible time in the equipment available and the emphasis is on the rate of production of the desired enantiomer. In a manufacturing process, the emphasis is on cost of the product (in $ per kg, etc) and this may change the way in which the process is run. Where the final product is valuable, the rate of production remains critical but cost considerations can result in a non-optimum process (from the chromatographic viewpoint) being preferred. For a production process it is worth spending the time to optimize the separation using all possible stationary phase and mobile phase combinations – and also to calculate the economic consequences of several options to determine the best. It is essential to test intermediates at all points in the synthetic process downstream of the introduction of the chiral centre where there is no possibility of racemisation in processes still further downstream to find the best point at which to run the chromatographic resolution. This may be self-selecting in some cases where the chiral centre is introduced late in the synthesis, while in others there can be a genuine best point at which to introduce the resolution. Although at the production scale the recovery of solvent can reach over 99.9%, the cost of some solvents (such as acetonitrile under the present economic climate) may influence the choice of one separation option over another.

At present, manufacturing scale SMB processes are generally outsourced to a CMO with this capability. There are several companies in the world with such equipment (eg Ampac, Daicel, Johnson Matthey, Novasep and SAFC) where large scale separations may be carried out. This is because one of the greater costs of SMB processing is the investment in equipment and infrastructure. If a new crystallization process is envisioned for a pharmaceutical product, there are usually sufficient tanks in a manufacturing plant to accommodate it. Most companies do not have SMB equipment in place and this extra investment can militate against implementation of a process even where it has longer term economic advantage. Another advantage of outsourcing such processes is that the CMOs have good experience in design, running and maintaining them which is not generally available.

Conclusion

Preparative enantioselective chromatography is a fast and efficient way to produce highly pure enantiomers from racemic (or enriched) mixtures. Where there is a critical need to prepare pure enantiomers in the shortest possible time (for example in the pharmaceutical industry from early discovery to the point where the product is moving through Phase 1 and perhaps Phase IIa clinical trials) the most effective route is generally through chromatographic resolution of the racemate. It is easy to develop a small scale separation of a few hundred milligrams of racemate and to progress to having operating conditions for isolation of kilogram quantities and even a production scale process within a few weeks. Once the first few kg of enantiomer have been prepared, the pressure to have material quickly is reduced so there is time, perhaps, to compare the chromatographic route with alternatives. This does not imply that enantioselective chromatographic processing is not used at the manufacturing scale; the imperative is to find the most cost-effective process. It should, of course, be remembered that the cost of chromatography for the first few grams of material is very different from that for the first 10 metric tons; the costs decrease quickly with scale and with further optimization of the separation process. Chromatographic processing should always be considered as one of the options for the manufacture of pure enantiomers.

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