recent developments in combinatorial chemistry

Combinatorial chemistry has advanced significantly from the early 1990s when it was a new science of mainly conceptual interest to today where it has evolved into a key component of the drug discovery industry. The needs of the Pharma industry have demanded that compound libraries produced by the techniques embraced by this technology are of significantly higher quality and 'value'. This article describes where recent advances have been made in this area from a service company perspective and how these have impacted on the increasing importance and indeed reliance of compound libraries to identify new chemical entities for the pharmaceutical industry.

By Dr Anthony D Baxter

The early days of combinatorial chemistry were characterised by enormous enthusiasm for a new technology that promised to create thousands if not millions of compounds for each chemist, each year and for each new screen target. These initial methodologies often involved preparing mixtures of compounds on solid phase bead-like materials. The resulting compounds not only consisted of numbers of different constituent molecules but they were not analysed to any great extent given the lack of suitable analytical techniques. When these materials were screened many 'active' compounds were found. However, the numbers of 'false positives' (compounds whose original activity could not be confirmed upon resynthesis) and the consequent confusion and loss of time led the industry as a whole to demand a review of the procedures of how such screening materials are prepared and analysed. From these early experiences, the concept of parallel synthesis of single compounds emerged which led to a great improvement of quality of screening materials.

This newer technology was embraced by the Pharmaceutical Industry and service providers alike and the numbers of sources for such compounds increased dramatically. The results of screening these materials, especially in the light of increasing complexity of screens and the sheer numbers of data points being obtained, still gave much room for improvement as the Pharma companies demanded further advances in production efficiencies and quality of materials. The last five years or so has seen the service industry, in particular, respond to these significant challenges and this article covers four areas that we consider to have made the greatest contribution to the desired advances.

Automation

Many companies (including ourselves) originally sought to solve the production efficiency and quality issues by purchasing 'state-of-the-art' automated synthesis stations. These expensive items of capital expense have complex liquid handling systems combined with multi-well reaction blocks that are capable of heating, cooling, shaking and blanketing reactions with inert gas for use in air-sensitive reactions. Not surprisingly, perhaps, such systems proved difficult to operate and to train staff to use. They were also prone to systems failure and required frequent overhaul and repair. It became clear to us that such systems are not the sole solution to the issues of efficient compound production although they still have their place for the automation of specific chemistries and compound types. Where automation has made an enormous impact to quality library production is in the areas of post-synthesis manipulations. In these areas, robots can operate virtually all the required translocational movements of materials. Such systems include splitting of mother plates into multi-daughter plates for analysis, cherry picking 'good' compounds from prepared libraries and formatting of compounds into storage vials or screening microtitre plates. Such systems can be readily purchased from manufacturers and require little or no customisation prior to installation. Where we have made significant improvements to production

efficiency is in the areas where there is no commercial solution. Collaborations with local engineering companies and robotic experts from academia have resulted in several systems that have been designed for custom applications. Three examples are in resin dispense (**Figure 1**), resin washing (**Figure 2**) and trifluoracetic acid dispense (**Figure 3**). All three applications have in common repetitive movements of potentially hazardous materials to relatively precise locations. The equivalent manual actions that preceded these automated developments raised issues of consistency of performance, repetitive strain and, in some instances, operator safety. The additional enhancement of running all the automated systems in an integrated and operator-friendly manner has helped to maximise performance efficiency in library production.

Production management

One area that has made results production of libraries more certain and consequently has improved their quality is the adoption of working practices learned from our colleagues in large-scale pilot plant production. Instilling the discipline and practices associated with GMP (Good Manufacturing Practice) of large scale batches of materials to producing compound libraries, especially large lead discovery libraries, has been reflected in much higher 'hit rates' (ie the numbers of compounds passing a predetermined specification), higher average purities of compounds, lower library failures and increased production efficiency. The sort of innovations employed are: use of SOPs (standard operating procedures), IOPs (internal operating procedures), IPCs (in-process checks) and quality control of every aspect of library production. This modern day approach contrasts markedly with the hit and hope approach most people originally employed. We adopt the flow scheme shown in **Figure 4** in all library synthesis campaigns.

Library production commences after the 'virtual library' (the designed but yet to be prepared compounds) has been agreed (in our case, together with clients) and the constituent chemical building blocks and reagents have been sourced. The second phase of production we term 'development' and this consists of defining the synthetic route that is going to be employed for production of the library. Given the increasing range of synthetic options for library production, for example, solid phase, solution phase, building block synthesis and resin capture, this phase is becoming increasingly complex. Once the development phase has been completed and signed off by a member of the management team, the process moves into 'validation'. This is a critical phase of production as it defines that the vast majority of the desired/agreed compounds can be accessed by the route identified in 'development'. This phase is typically the longest in

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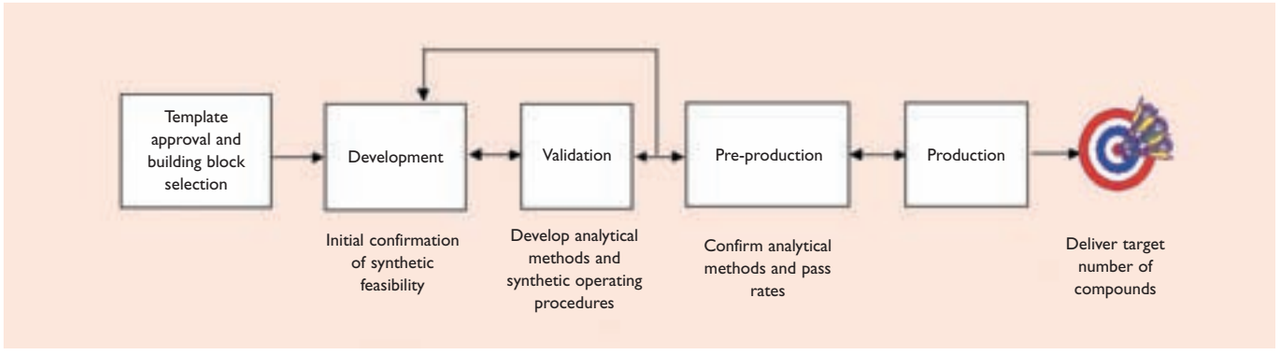


Figure 4
Oxford Asymmetry International adopts this flow scheme in all library synthesis campaigns

terms of man-hour effort and our experience has been that the more effort that is put into 'validation' the more successful the final library proves to be. Validation also serves to identify which analytical methods are most appropriate for each particular library and SOPs are drawn up for the next phases of production. In some cases, 'validation' indicates that the desired library cannot be prepared using the route identified in 'development'. In these cases, the library production process is halted and returned back to development (with the approval of the client) for an alternative route to be identified. In years past, we used to go straight from a successful 'validation' phase into full scale production. On several occasions, production runs failed. This was attributed to the fact that 'validation' tends to be micro-managed with much attention to detail whereas 'production' tends to be heavily automated with less 'hands on' time being employed. Consequently, production chemistry must be robust enough to cope with the rigors of the production environment. We solved this problem by incorporating a phase we term 'pre-production'. In 'pre-production', some 5-10% of the final library, using the most diverse of the synthetic inputs, is prepared using the identical automation and methods to be employed in the full production run. Examination of the results of this 'pre-production run' gives an insight into the predicted 'hit-rate', delivery times and potential hiccups to be expected in the full potential run without committing the full resources should a major problem be identified. We have found that by employing the above working practices, our predicted success rates for delivery of compound libraries to clients has dramatically improved. While these practices have most application to the large lead discovery libraries, we also apply the basic principles to the higher value per compound lead optimisation libraries. It should be stated that such production mentality constraints does not often sit comfortably with experienced medicinal chemists and training of operational chemists who are more attuned to the world of GMP, TQM and QC is equally important to a successful operation.

Access to building blocks

One of the original imaginings of solid phase 'combinatorial' chemistry was that any given reaction involving every possible synthetic input would proceed to completion, and thus, from any combination of reagents the entire 'virtual' library would be successfully prepared. This pronouncement remains sadly elusive and many combinations of reagents in otherwise well validated chemistry do not yield the expected products. As a result of this, in the early days of library synthesis, there were times when relatively small portions of virtual libraries were accessed. This caused such libraries to consist of similar looking molecules which were devoid of true structural diversity. One successful solution to this problem that we enthusiastically adopt is the use of pre-synthesised building blocks in library production. Such building blocks are prepared, generally using 'traditional' solution phase methods, but are prepared rapidly, on large scale (typically 100g-3kg) and to meet the desired structural characteristics demanded by the client. The preparation of building blocks requires a different skill set to combinatorial synthetic chemistry particularly large-scale chemistry, process research experience and an appreciation of experimental reaction design. Such dual skill sets are rarely found within the service industry. We are fortunate enough to have a dedicated team of some 10 scientists who prepare several hundreds of building blocks for our production teams to incorporate in their libraries. We have found that access to these materials has several advantages. They allow the incorporation of certain sub-structures into libraries that are inaccessible using parallel synthesis limitations. Proprietary building blocks afford structurally novel compounds for screening (and potentially an improved intellectual property position) and help avoid me-too type compounds that are more universally available. Design and synthesis of building blocks as central templates affords the opportunity to prepare focused 'drug-like' libraries which can be aimed at specific protein (enzyme, ion-channel or receptor) targets. Such libraries have real added value to both client and service provider alike.

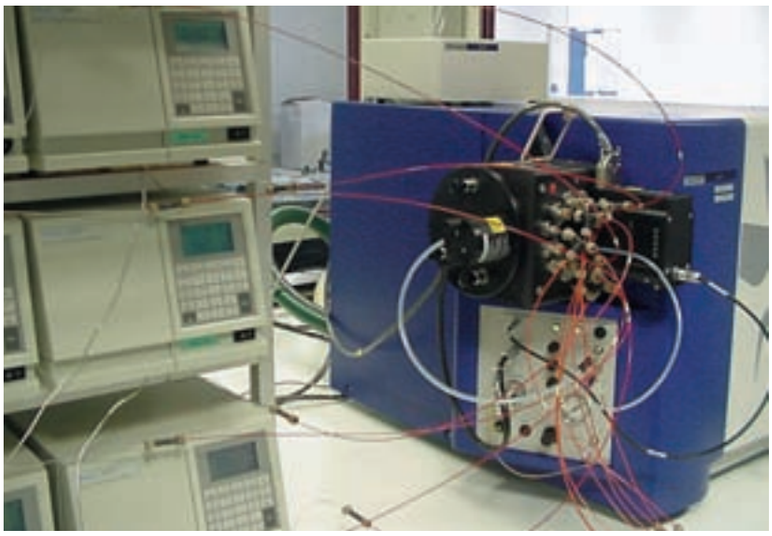


Figure 6
The MUX from Micromass

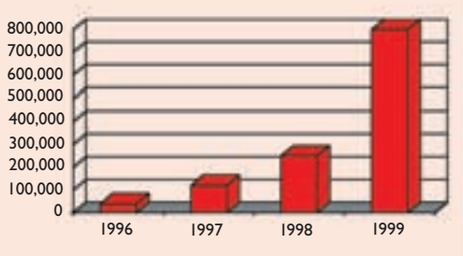
Analysis

The first commercially available compound libraries were subjected to relatively scant analytical interrogation. Typically only 5-10% of the library was examined and then by mass spectrometry (MS) only. The past five years have seen this picture change from being an optional extra to becoming an integral part of determining a library compounds synthesis and properties.

In the mid 1990s, analytical methods had improved such that the majority of compounds were analysed by MS and, separately, liquid chromatography (LC). Analysis of library compounds became easier as the cost of MS interments was reduced and reliability and ease of operation improved. Other advances in technology introduced alternative LC detectors. Evaporative light scattering detectors have become commonplace in the industry and these mass-based detectors are capable of quantifying components using properties other than UV absorption (the most widely accepted technique). However, these advances did not solve the key problem of determining the absolute purity of a new library compound without a standard, pure material.

One of the problems of such an analytical approach was that, occasionally, the MS signal and LC trace did not match and compound structure and or purity was mis-assigned. The other major problem was that compounds were being prepared in huge numbers due to the 'industrialisation' of the process (see **Figure 5** for Oxford Asymmetry's experience). To cope with such numbers of new compounds requiring analysis more rapid analytical techniques particularly in LC became essential. Use of ballistic gradients became standard and run times per compound were reduced to two minutes or less. The trend of using stand-alone LC and loop injection MS in mode became the standard, most cost-effective method of analysing large lead discovery libraries. The most recent development is the use of coupled LC-MS whereby each compound peak is analysed in the sample and compound purity and structure can be unambiguously assigned. This technique typically suffers from a low throughput, but a very recent development is the use of parallel injection ports from up to eight LC machines into a single mass spectrometer. Such an instrument, MUX, from Micromass is shown in **Figure 6**. This most recent technology has finally solved the seemingly impossible problem of analysing, in a truly qualitative way, large numbers of compound in synchrony with their synthesis. For example, the purity and identity of all the products in a 96-well plate can be completely determined using LC-MS in under an hour.

Figure 5
Number of compounds analysed



Summary

The development of new technologies to assist in the production of high quality compound libraries continues apace and it is interesting to note that as one bottleneck is solved in one aspect, another bottleneck arises elsewhere. Nevertheless, the past five years have seen a remarkable transformation in the capabilities of companies to produce high quality libraries which are now being screened and actively utilised in the drug discovery process. The high standard of libraries available in terms of their design, 'drug-likeness' and quantity represents real value for the pharmaceutical industry. DDW

Dr Anthony D Baxter *is Chief Scientific Officer for Oxford Asymmetry International Plc and has been with the company for five years. He obtained his PhD from the University of Salford working with Professor Stanley Roberts in prostaglandin synthesis. He commenced his career at Glaxo Group Research in 1983 as a medicinal chemist and became Research manager at Ciba-Geigy Central Research in 1990 before taking up his current position.*