Successful implementation of automation in medicinal chemistry

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Automation in medicinal chemistry is often seen simply as a part of the combinatorial chemistry technologies used to meet the need for large, diverse screening collections for lead generation. However, the application of automation to the lead optimization phase of drug discovery offers the prospect of reduced cycle times via increased efficiency in target compound preparation. The realization of this goal requires the integration of efficient processes with equipment capable of delivering quality compounds – and, of course, the skilled medicinal chemists.

Although automation is used extensively in many areas of industry, and right through to the domestic situation, its application to medicinal chemistry has not been particularly marked[1]. Traditionally, the medicinal chemist designed and synthesized series of individual pure compounds in the search for drugs with useful biological properties using a wide range of methods that often included many synthetic and purification stages. The synthetic part of the medicinal chemistry process has therefore tended to involve highly skilled handcrafting of compounds, with the emphasis on the preparation of a few specific compounds rather than large numbers of compounds. Consequently, the output of medicinal chemists in the 1970s was expected to be only 20–25 compounds per year. Since then, the advent of genomics and high-throughput screening has created a demand for larger numbers of compounds to screen in order to identify hits for new biological targets and to develop leads to new drugs. In response to the call for more compounds, many companies have introduced combinatorial technologies[2] into their chemistry effort, and with this has come significant automation[3]. However, much of the earlier combinatorial effort was based on solid-phase peptide chemistry[4], in which amide bond formation has been highly optimized for the preparation of peptides, and on the synthetically more efficient preparation of large libraries of compounds as mixtures[5]. This is in contrast to the wide range of chemistry and yields involved in the synthesis of single, small organic compounds of greater relevance to medicinal chemistry.

The aim of most automation is to improve efficiency, not just in terms of simple output per head, but also as measured by increased product quality and the reliability and reproducibility of the process. In chemistry, the application of automation to combinatorial technologies to facilitate the preparation of large numbers of compounds can deliver increased efficiency. However, the real challenges for automation in medicinal chemistry lie in its acceptance outside of specialist groups and its application to the wide range of chemistry used by medicinal chemists[6]. The application of efficient automated methods to the lower throughput, higher value medicinal chemistry effort will contribute to a reduction in cycle times for the drug discovery process and will allow more drug targets to be investigated.

Basic issues for automation in medicinal chemistry

There are a large number of issues that need to be considered for the practical implementation of automation into

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 1359-6446/00/$ – see front matter PII: S1359-6446(00)01571-3

medicinal chemistry. First, the equipment must match (within reason) the most demanding chemistry that it is expected to handle, and at the appropriate scale and throughput. The reliability of the equipment is critical, but this is very difficult to evaluate for the many newer products and even for established ones because the user base can be relatively small. Simpler modular equipment might offer greater flexibility and be more reliable than some complex self-contained units. Further, cost is an important issue, particularly as the figures involved can be large compared with those normally spent by medicinal chemistry groups, but the expenditure has to be viewed as an investment for improved productivity[7]. The efforts to contain costs by opting for cheaper equipment is short-sighted if reliable performance is sacrificed, and the negative impact of downtime for automated equipment is potentially far greater than for traditional laboratory equipment. The duplication of equipment might be required for the more critical parts of the process. Almost any process can be automated, but unless continuous operation is necessary, it might be more practical and economical to concentrate on the most intensive parts of the process even if this means that some manual intervention is still necessary. A great deal of automation operates in a serial fashion, which can lead to slow processing, but this can be improved if operations can be carried out in parallel (which is also true of many manual processes).

The laboratory requirements for automated and robotic equipment tend to be different from those of traditional chemical laboratories. For example, much of the equipment is larger and either does not fit into standard fume cupboards or its bulk prevents the hood from operating efficiently, and therefore different levels and methods of containment might be appropriate. Also, more space is required for processing and safe storage (of reagents, consumables, products and solvents), and associated computing equipment has to be accommodated so that it can be used comfortably without causing obstructions. In addition to the location of equipment, the target user group(s) also have to be considered, with the two extremes being a specialist group vs widespread decentralized use. In the short term, a specialist group can be very efficient, but to leverage the wider benefits of automation across medicinal chemistry it is essential to have a wider user base and to encourage the integration of automation into medicinal chemistry. Efficient data management[8,9] is integral to effective automation in medicinal chemistry, for example, traditional methods of record keeping, registration, labelling and submission to screening cannot cope with the output from automated array synthesis.

Finally, it has to be accepted that in the implementation of automation in medicinal chemistry, it is difficult to consider all of the issues, and that there is no single correct approach. This is particularly true because of the rate of change that has occurred over the past few years with evolving requirements and capabilities, and the availability of new equipment. Risks have to be taken in selecting equipment and technologies, and it is important to avoid the situation of 'evaluation stasis' in which new developments invalidate any evaluation, so that it has to be revisited *ad infinitum*, and in the meantime the benefits to be gained from the use and experience of new equipment are lost. Successful implementation depends less on the choices made than on the commitment made to make them work.

When applying automation to a synthetic process, there are some simple common-sense guidelines. The first is to choose the instrument that can carry out the particular chemistry and produce the required quantities and numbers of compounds. Ultimately, the ability to automate chemistry is limited by the capability and compatibility of the instrumentation. For example, at SmithKline Beecham (SB) there are devices capable of performing high-throughput chemistry and high-performance chemistry in both solution and solid phase[10]. (Hird, N. and MacLachlan, B. High-throughput automated organic synthesis using the Myriad Core System. *International Symposium on Laboratory Automation and Robotics*, 18–21 October 1998, Boston, MA, USA.) The decision to automate the use of solid phase vs solution phase techniques ultimately depends on the chemistry being performed. However, in general, solution phase techniques have been used for shorter reaction sequences in order to avoid solution phase work up and evaporation, which can be required at each step, although pioneering work with solid supported reagents is redressing this bias[11]. Solid phase chemistry is thought to be more suitable for longer reaction sequences because only resin filtration and washing steps are required for reaction work up. For trial reactions, it is better to start with proven chemistry and then select the most appropriate robotic conditions, rather than vice versa. However, traditional chemical processes are likely to need modification before transfer to a robot, for example, it is much easier to handle isolation by extraction and evaporation rather than by filtration of crystalline solids. Validation of the whole process needs to be completed before it is committed to production and analysis (as with a traditional synthesis) is an essential part of the process. There are occasions when it is not advisable to automate chemistry. For example, when developing new chemistry (although process development can benefit), if this is beyond the capability of the

instrument, and if long multistep sequences or prolonged reaction times are involved. By contrast, it can be entirely feasible to automate a process, but there might be no advantage in doing so, for example, when small numbers of products are proposed or for one-dimensional arrays.

Equipment and technologies used at SB

Starting from the purchase of a single ACT 396 (Louisville, KY, USA), we have developed a process based on a flexible set of automation tools able to deal with a range of chemistry both in solution and on solid phase. Importantly, post-synthesis as well as synthesis tools were included. The first tier of synthesis tools included a range of equipment allowing flexibility. Thus, a Zymark solution synthesizer (Hopkinton, MA, USA) has moderate chemical capability but is able to provide several grams of each product as reagents for use in other robotic and manual systems. The Bohdan RAM™ (Bohdan Automation, Vernon Hills, IL, USA) is also a solution synthesizer, which operates on a smaller scale but has better chemical capabilities and is particularly attractive for structure–activity relationship (SAR) work.

A variety of older ACT systems, suitable for solid and solution phase synthesis, can be used for library production and for the preparation of directed arrays, but are somewhat restricted by their non-modular format. Irori (San Diego, CA, USA) technology makes use of normal equipment (e.g. round-bottom flasks) making it 'chemist friendly' and is used for larger (solid phase) arrays[12]. Although essentially a manual mix and sort process, automation is necessary to facilitate the synthesis of larger numbers by the Irori technology, particularly at the sort and wash stages. Finally, there is the Myriad® Core System (MCS™; Mettler-Toledo Myriad, Melbourn, Hertfordshire, UK), an example of the new generation of synthesizer, which is suitable for the most demanding high-throughput solution and solid phase chemistry. The MCS is a large modular unit with 192 glass reaction vessels, which have a novel twist-cap seal to allow septum-free access in an inert atmosphere, and are independently processed in sets of 48. Special purpose modules, for example, for rapid resin washing and liquid–liquid extraction, have been developed to extend the capabilities of the MCS. A key feature of the MCS is the availability of the 24-vessel Myriad Personal Synthesizer (Myriad PS™), which uses common hardware and software for validation and lower-throughput work that can be readily transferred to the MCS. Myriad Personal Synthesizers are currently being installed in all of the medicinal chemistry laboratories in SB.

After synthesis, work up may be needed, for example, the Hamilton 2200 (Reno, NV, USA) can be used for automated solid phase extraction (SPE), and a Biotage Parallex™ (Charlottesville, VA, USA) can provide the main preparative HPLC purification system. Liquid sampling and transfer are accomplished with Tecan Miniprep™ (Hombrechtikon, Switzerland) and Hamilton MLS™ systems and evaporation is achieved by centrifugal vacuum systems from GeneVac (Ipswich, Suffolk, UK). All products are quantified by mass using Bohdan Automated Weighing Stations and are subjected to quality control (QC) by LC–MS using Micromass OpenLynx Diversity™ (Manchester, UK) and Finnigan aQa™ (Manchester, UK) systems for high-throughput analysis, with Agilent Technologies MSD systems (Stockport, Cheshire, UK) used to support validation and route development. At the end of the chemists' involvement, the in-house Registration, Analysis and Design Interface for Combinatorial and Array Libraries (RADICAL) software package is used, for example, for label printing and the electronic generation of files for registration into the corporate database and of dilution tables for solution preparation in screening (Calvert, S. Managing the combinatorial chemistry process and information. *MDL US User Conference*, 16–19 May 1999, San Francisco, CA, USA). The importance of good-quality information technology (IT) products and services to enable effective automation in medicinal chemistry must be emphasized. Most large pharmaceutical companies have proprietary systems tailor-made to suit existing in-house databases and processes. However, commercial systems are available[8,9].

The specialist core group and open access

The approach taken at SB was to start with a dedicated core group, the Combinatorial and Chemical Technologies Group (CACT), and then to add a large open-access component. Members of CACT concentrated on the use and development of particular components in the automated synthesis process, and it must be emphasized that considerable focus was (and still is) required in order to transform many new items of equipment into effective, usable tools. These 'super users' have a greater awareness of instrument capabilities and new developments, are able to develop in-house modifications and innovate in response to new challenges, and provide training to others. Their greater technical experience enables them to carry out some maintenance and troubleshooting, which reduces dependence on the supplier, but at the same time facilitates the building of good relationships with suppliers. The successful open-access usage of automation equipment by medicinal chemists was made easier by having modular systems, in which individual pieces of equipment perform simple tasks well, and by having several synthesizers to cope with a range of chemistry (both solid and

solution phase). It is also important, in order to facilitate training and problem solving with multiple users, to standardize processes where possible. Thus, generic routines for equipment operation were developed and all chemists used a standard array synthesis process.

The approach taken by SB has clear advantages. A range of equipment allows for flexibility so that different targets and chemistry can be handled, and components in the process can be upgraded or replaced with new instruments as necessary. The inclusion of medicinal chemists is important in that it is vital to integrate combinatorial and automated methods into their planning, rather than adding it as an afterthought. Finally, open access enables greater use to be made of the equipment than could be achieved by a small core group acting alone, and this has led to significant productivity gains. Indeed, during 1999, greater than 30% of leads actively progressed at SB came from high-throughput chemistry.

Training and integration with medicinal chemistry

To assist in the successful implementation of automation in medicinal chemistry at SB there has been an extensive programme of training and integration. In addition to the normal movement of staff between groups, the interchange of personnel between CACT and the other medicinal chemistry groups has been encouraged. This is not always easy because the movement of key staff can have a temporary detrimental effect on group performance and some might not want to change jobs. However, these changes are important for the company and for many staff they represent a valuable career development opportunity.

More frequently, temporary transfers were used as part of a programme in which medicinal chemists were seconded to CACT, usually for a six-month period, during which time they worked on combinatorial targets linked to their medicinal chemistry programme. This allowed them to focus on the use of automation and combinatorial methodologies without the competing pressures of programme work, and was an excellent vehicle for building links between the scientists from different groups. The nature of this training programme meant that it was restricted to approximately eight chemists per year, but it was complemented by a short intensive programme, open to all, which was able to accommodate 48 chemists per year. This intensive training served the dual purpose of utilising SB proprietary reagents for the synthesis of libraries and exposing medicinal chemists to automated methods. To accomplish this, chemists from the medicinal chemistry programmes spent two days (on average) in CACT and followed a standardized high-throughput solution phase synthesis protocol to prepare small arrays of compounds. This exposed them to the array process: design, automation, SPE, high-throughput analysis for QC and registration. Automated equipment was used for the synthesis, purification (SPE), weighing, preparation of analytical microtitre plates and high-throughput QC, with RADICAL used throughout the process from design to registration. During 1998 and 1999, this effort produced a significant number of compounds, but the most important point is that chemists were encouraged to apply automated methods to their own work following this introduction to the potential applications of automation.

The core group has made extensive use of Irori technology to prepare targeted and lead generation arrays containing up to ~10,000 compounds. However, this approach to combinatorial chemistry is also attractive to medicinal chemists, particularly for lead explosion and deconvolution of array members or mixture components previously prepared on solid phase, but they have also applied this technology more widely using MiniKans™ as well as MicroKans™. Extensive automation is not required for synthetic steps because of the numerical efficiency of parallel processing, but is advantageously applied elsewhere in the process, especially as array size increases and/or more synthetic steps are involved. Thus, the Accusort 10K™ is used for the unattended sorting of reactors at each step and for archiving before resin cleavage, in what would otherwise be slow and tedious processes, especially for larger arrays. Multiple washing of reactors, which can be physically demanding, is required at each stage and Irori have developed an automated washing station; SB has its own smaller design, which might be commercialized. The loading of resin into MicroKans can be a very time-consuming process and automated resin dispensers are available that might be particularly useful for filling large numbers of Kans with the same resin. However, a parallel filling process allows for the efficient manual loading of resins using equipment based on an SB design and available from Irori and Radleys (Saffron Walden, Essex, UK).

Many of the medicinal chemists have applied the general methodology of the high-throughput synthesis training programme to their own work, often very successfully. However, this type of synthetic protocol is not always appropriate to the needs of medicinal chemists, especially to follow up leads originating from file compounds made by traditional solution phase methods, and a robotic synthesizer such as the Bohdan RAM synthesizer might be more suitable. The RAM is capable of high-performance chemistry with a liquid–liquid extraction capability on a scale (up to ~100 mg) that provides sufficient compounds to conduct secondary studies and the moderate output

from the 48-vessel array is well suited to focused SAR libraries. Often the known solution phase methods can be adopted with only minor modifications for use on the RAM. This system has been used to excellent effect by medicinal chemists and proved so popular that it was transferred from the core group into medicinal chemistry.

The key advantage of the application of automated methods by the medicinal chemist is that the broad SAR patterns can be identified quickly, and this in turn helps to reduce the cycle time spent in the research phase of discovery programmes. As new facets of SAR are identified, they in turn can be quickly assessed against a range of structural features by the preparation of more focused arrays. Significant progress can be made from the preparation of just a few arrays, typically totalling from tens to hundreds of compounds, rather than the many thousands of compounds in lead generation arrays. Although the array format is often viewed as a restriction, in reality it allows the medicinal chemist the freedom to make more (different) compounds more quickly using automated methods, provided that they seize the opportunity. This has resulted in some unexpected results when arrays have been extended beyond the narrow medicinal chemistry rationale, such as high potency from new combinations of an unfavourable component, to the generation of leads for other unrelated targets.

Post-synthesis: automation in analysis and purification

Traditionally, the medicinal chemist was expected to purify single compounds by recrystallization and column (latterly flash) chromatography, although others usually carried out analysis and the more specialized purification techniques. With advances in instrument automation, it has now become routine for medicinal chemists to run their own MS and NMR spectra using open-access instruments, and this trend will continue. Evidence of this at SB is the replacement (in open-access mode) of MS by the much more powerful tool of LC–MS.

A key component of any high-throughput production process is QC, and automated analytical instrumentation has advanced rapidly to meet these needs. Micromass OpenLynx Diversity and Finnigan aQa LC–MS systems can be operated almost continuously using microtitre plate sample input and with efficient high-speed chromatography have a throughput of about 500 samples per day per instrument. The results are processed electronically to generate a pass or fail summary based on an association of the expected molecular weight with a UV peak area meeting the QC criteria. Chemists browse on-screen to check results where necessary. With this type of instrumentation, collecting the data is relatively easy, but the challenge is to improve the data processing to cope more reliably with diverse samples run at such high rates. In addition to the dedicated LC–MS instruments for high-throughput analysis, which are run by a specialist group, extensive use is made of additional automated instruments, such as Agilent Technologies MSD and Micromass units, run in open-access mode for array validation work, low-throughput QC and general synthetic work. Evaporative light scattering (ELS) detection[13] is also used with LC–MS and has the advantage over UV detection of a more linear mass response.

NMR spectrometers have been used routinely with autosamplers and automated operating routines by medicinal chemists in open access mode for some time, but more advanced high-throughput automation is run as a service. The latter is based on the Bruker AVANCE 400 NMR equipped with Bruker's Efficient Sample Transfer (BEST) accessories system (Bruker Analytik GmbH, Rheinstetten, Germany). This utilises a flow probe and automated sample injection directly from a microtitre plate to give a throughput of 15 samples per hour and excellent proton spectral quality on samples of 1 μM. For example, this system is used for obtaining spectra on arrays for SAR studies where additional confirmation of structural integrity is required. The results are returned to the chemist electronically and viewed on a browser, but data handling for large numbers of compounds is more challenging than with LC–MS.

The medicinal chemist and analyst now have to work more closely together because they are operators in a single continuous process. The chemist prepares microtitre plates using an automated liquid handler and passes these to the analyst together with electronic data files containing, for example, expected molecular weights of the products. The results are returned electronically to the chemist, who uses the automated data analysis (in the case of LC–MS) to trigger the registration process for those samples that pass the QC criteria.

The purification of large numbers of compounds produced by high-throughput array chemistry might be necessary because they either fail the normal QC criteria for screening or a uniformly higher quality product is required for SAR studies. To meet this need, CACT have two fully automated Biotage Parallex high-throughput preparative HPLC systems, each running four columns in parallel with UV-triggered fractionation. When used for array samples in the 10–30 mg range, the throughput is 200–300 samples per instrument in an average working day. Key to the successful application of these instruments is to take a pragmatic rather than an analytical approach, and to reduce

the burden of post-purification processing and attendant liquid handling. The fraction collection criteria (slope, threshold and wavelength) are adjusted to allow 'Intelligent Fraction Collection'[14], which reduces the number of fractions collected, and in addition LC–MS data for the crude samples can be used to guide fraction collection. Subsequently, 'Intelligent Fraction Selection' is carried out with the aid of Winnow, software developed at SB that reduces the number of fractions selected for analysis based on absorbance, volume and LC–MS data of the crude sample[14]. Winnow is also used for data transfer between the Parallex, liquid handlers (for transfer of selected fractions for QC or evaporation) and the Finnigan aQa LC–MS. As an example of the purification process, 4320 compounds from an 11-step solid phase synthesis were purified on a Parallex to give 3–4 fractions per sample, of which 1–2 were selected for analysis by LC–MS and finally 4012 samples (93% of the total, with >90% having >95% purity) were submitted for screening. The Parallex has been widely used by members of CACT and by medicinal chemists for the purification of lead generation and optimization arrays. The success of this type of instrument highlighted the need for instruments to carry out the same task for the smaller arrays and single compounds more typical of medicinal chemistry.

Current developments in automation at SB – the future of medicinal chemistry

The developments mentioned above, from the introduction of the first ACT into the core group through to the installation of Myriad Personal Synthesizers into medicinal chemistry laboratories, have occurred in just three years. Developments are still underway and we need to review the current situation and the plans for the next 1–2 years.

In the medicinal chemistry laboratories, the installation of personal synthesizers has been augmented by the addition of Remote Incubators for increased throughput (Mettler-Toledo Myriad, Melbourn, UK), instruments for reaction work up [e.g. ALLEX™ Automated Liquid–Liquid Extractor (Mettler-Toledo Myriad, Melbourn, UK)], GeneVac HT4ii evaporators and Biotage Quad 3 automated parallel flash chromatography apparatus to form the core of Automated Medicinal Chemistry Workstations. Because of the common platform, chemistry from the personal synthesizers can be transferred directly to the MCSs for the production of larger lead explosion or lead generation arrays. The majority of the output from the Automated Medicinal Chemistry Workstations will be for SAR work and purification is likely to be required for some of the products. To address this need, SB collaborated in the development of the Biotage Flex™ system. Unlike the Parallex, this is designed to be an open access, walk up version for medicinal chemists with 1–4 independently operated columns available. Analytical requirements are likely to be met by increased local availability of open access LC–MS, whereas NMR of arrays will be provided most efficiently from flow-probe instruments. Automated liquid handlers will also be required for the preparation of analytical and purification plates and for reformatting. Some modification of existing medicinal chemistry laboratories will be required along with changes in work practices. Although the local Automated Medicinal Chemistry Workstations might function under the control of local experts, all medicinal chemists will have access to and make use of the equipment.

In addition to the localized automation facilities, which will meet the majority of the medicinal chemistry requirements, a centralized high-throughput unit ('compound suite') will be established for the highly efficient production of compounds for lead generation. This unit will house the major synthesis platforms (e.g. MCSs, Irori, microtitre plate systems) along with reagent storage and handling, purification (Biotage Parallex, with liquid handlers and plate hotels for automated post-purification processing), analysis [high-throughput LC–MS using MUX™ (Micromass, Manchester, UK), BEST NMR], and automated weighing, labelling and sorting workstations (Bohdan). A key design concept for the new building is that of a large flexible workspace with drop-down services to allow for the deployment of new technologies with minimal alterations, in contrast to the rigid highly serviced design of traditional synthetic laboratories. While this building is under construction, a smaller experimental unit will be used as a test bed for the evaluation of design concepts, the key elements of the production unit and the operation of an efficient production process. An essential feature of the operation of a complex production process will be the ability to track the progress of work and to schedule the use of individual workstations and robots. To achieve this, the software package ACE (Automated Chemical Environment) is under development with The Technology Partnership (Melbourn, Hertfordshire, UK) and will also be evaluated in the interim facility. (Calvert, S. Managing the combinatorial chemistry process and information, *MDL US User Conference*, 16–19 May 1999, San Francisco, CA, USA.)

Conclusion

The successful implementation of automation in medicinal chemistry requires the dedicated efforts of a multi-skilled team. Capital investment for equipment, software development and laboratory facilities must be undertaken.

This investment is necessary to leverage the expertise and to harness the potential productivity of general medicinal chemists if the pharmaceutical industry is to continue to prosper.

Acknowledgements
We would like to thank their colleagues at SB for their commitment to high-throughput chemistry and laboratory automation.

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