IMPROVING ADME SCREENING PRODUCTIVITY IN DRUG DISCOVERY
INTRODUCTION

Improving the quality of the decisions made at the drug discovery stage can profoundly impact the efficiency of the drug discovery and development processes thereby allowing better decisions to be made earlier. The synthesis of large compound libraries has resulted in the rapid screening of multiple compounds against disease targets. Compounds registering a hit are ranked based on their activity, binding, and specificity. This alone however, is not sufficient to select a good drug. Compounds with undesirable physicochemical properties which affect ADME, must be eliminated from the candidate selection process as early as possible to allow time and effort to focus on more promising candidates. Currently, experiments including solubility, chemical and biological stability, water/octanol partitioning, PAMPA, Caco-2, and protein binding are used to generate physicochemical profiles of the compounds under evaluation in drug discovery. In turn this data is used to aid the selection compounds for further development.

Enabling technologies such as liquid chromatography, mass spectrometry, and application-specific software now make it feasible to screen compounds based on their physicochemical properties earlier than ever before. By decreasing the time and labor involved with sample analysis and data interpretation, chemists in drug discovery laboratories now have the ability to:

- Prescreen chemical libraries for favorable physicochemical characteristics before performing expensive and time-consuming target affinity screening
- Streamline physicochemical analysis in lead optimization studies

Prepare

The measurement of physicochemical and ADME properties from these studies is easily enabled using chromatographic separation and quantification using LC/MS/MS. While the sample analyses may be efficient, developing the analytical method, processing of the data and the interpretation of the results often requires tedious and time-consuming manual manipulation and calculation. Simplifying and improving this process will allow higher throughput and a quicker turn around of results.

The initial assay work necessary for most physicochemical assays is carried out in 96-well microtitre plate. This format is amenable to automation, enabling much of the sample generation work to be carried out using robotics.

The typical analytical work-flow is outlined in Figure 1. We can see that a significant amount of scientific knowledge and analytical skill is required to effectively manage the process of LC/MS/MS method development and sample analysis. As the modern discovery ADME screening laboratory may see 50 to 100 or more new compounds a week, a faster more efficient process is required. To that end, tools available with MassLynx™ Software, such as the QuanOptimize™ Application Manager, have been developed to simplify, streamline and automate the workflow of ADME laboratories. It is applicable to any environment with high throughput quantification and is designed to streamline the workflow for hundreds of compounds through automation of:

- SIR or MRM method development and optimization
- Peak selection and integration
- Analysis and Quantification
- Reporting

Figure 1. The QuanOptimize workflow.

QuanOptimize has been designed to adapt to the user’s environment. It can run each of these functions individually or in a fully automated manner to suit the desired laboratory work-flow. QuanOptimize is ideally suited for situations with:

- High numbers of compounds
- Low numbers of analyses per compound
- Generic LC methods
- Optimizations in multiple ion modes are necessary
- Automation from sample optimization through to analysis and reporting is necessary

The software is driven by an easy to use wizard that can be operated in a true open-access mode where all the scientist is required to do is input the compound mass or elemental composition, supply the...
sample list, and place the samples in the autosampler. The instrument will operate under full automation to generate the method, analyze the samples, and report the final sample concentrations to the submitter by email. This removes the need for a skilled MS operator to manage the system and frees these experienced scientists to work on more complex problems.

The first option gives rise to analysis times in the region of 5 to 6 minutes, but reduces the overall peak capacity of the separation. The second option of monolithic columns produces 1 to 2 minute analysis times but requires very high flow rates and flow splitting into the mass spectrometer, thus reducing sensitivity and producing a significant amount of solvent waste. The sub-2-µm particle LC approach has the benefits of fast chromatography (1 to 2 minutes), increased performance and less solvent waste than traditional LC.

The combinatorial revolution in the mid to late 1990s was aided by the development of techniques such as parallel compound synthesis. This in turn led to an explosion in number of compounds generated for activity and ADME screening. While today’s more targeted approach does not produce nearly as many samples as CombiChem did, a large number of samples still need to be analyzed. This increased number of samples produced places a great strain on the DMPK analytical capacity. In order to address the need for fast sample turnaround and to aid informed decision-making, the analytical throughput of the laboratory must be addressed. As the rate-limiting step in the LC/MS process is the chromatography time there are two easy solutions; 1) reduce the analysis time, and 2) increase the instrument capacity. The second solution would require more laboratory staff, more space, and more instrumentation all requiring large capital investment. The first option is the most attractive. The chromatography time can be reduced in one of three main ways:


The Waters® ACQUITY Ultra Performance LC® (ACQUITY UPLC®) System was designed to take advantage of these small particle columns. More than 400 scientific publications, in just five years, have reported the increase in throughput, sensitivity, and resolution obtained with UPLC, making it the inlet of choice for mass spectrometry.

In addition to the fast data capture rate, the ionization mode (APCi and ESI) ESCi® and polarity can be switched in 20 ms. In ESCi, both ESI and the APCi modes can function alternating at MS switching speeds in both positive and negative polarities. This enables QuanOptimize to find the best mode of ionization in one experiment (Figure 2) as well as facilitating the detection of acids and bases in the same analytical run, again improving productivity.

ACQUITY UPLC are Xevo TQ MS are a unique combination providing high sample throughput without compromising either chromatographic resolution or MS/MS sensitivity.
Interpret

These new, faster analytical technologies result in the generation of a large volume of data quickly. The task of managing the data and deriving answers from the analytical results can at times be more challenging than the actual generation of the data. There are often several steps involved in the process: integrating the chromatograms, quantifying the samples, transferring the results to a second data spreadsheet, applying a formula to calculate the result (such as protein binding), and ranking the compounds according to the final calculation.

In order to process and review the large amount of data generated, Waters has introduced a dedicated software package, ProfileLynx™. Compounds can be analyzed and the data from different assays brought together in one efficient data visualization package. The results can be exported to third party (in house) data management systems in a facile manner for company-wide use and review.

Figure 2. 16 of 32 chromatograms (4 each of ES+/-, AP+/-) collected during single injection by QuanOptimize.
ProfileLynx handles sample set management, data processing, and results browsing (Figure 3) to simplify physicochemical property profiling. It provides exporting capabilities and interactivity with other software packages. The software allows for determination of:

- Solubility
- Stability (chemical and biological)
- Protein binding (on plates or columns)
- Membrane permeability
- Partitioning coefficients
- Chromatographic hydrophobicity index (CHI)
- Immobilized artificial membrane (IAM) column binding

The use of this software not only simplifies the process and removes the opportunity for transcription errors, but also dramatically cuts the time taken to process and interrogate the results, allowing more compounds to be processed per day. The effect on laboratory ADME productivity was measured in a real life situation. The results showed that the office time to transfer the results and data evaluation time were significantly reduced, dropping the overall processing time for one batch of samples from over 50 hours to just 20 hours (Figure 4).

Managing, storing and archiving can seem as daunting a task as running the samples and processing the data. The data created also needs to be sorted, collated, and finally shared with other scientists or teams such that they can act on the results. NuGenesis® SDMS facilitates the storage of the raw data, processed results, compound ranking, and other supporting material in one place, regardless of the kind of data generated. The software can even capture printouts and scan paper documents. The data is automatically catalogued at the time of capture, making it immediately accessible and sharable. This improves collaboration on data right from the beginning.

Once the data is archived, it can be used to generate reports. Using Forms with SDMS Vision Publisher™, even summary reports can be automatically generated. As well, the software reports can contain almost any type of data, from chromatograms and spectra to spreadsheets and pdfs. These reports will have direct links back to the data in the SDMS system. This versatility allows for easy collaboration across large organizations.
CONCLUSION

The combination of ACQUITY UPLC and Xevo TQ MS instruments along with MassLynx, QuanOptimize, ProfileLynx, and NuGenesis SDMS software provides a fast simple way to screen candidate pharmaceuticals for their ADME properties and their likely success as a drug (Figure 5). The use of these technologies linked together allows for a simplified workflow removing the majority of the manual user process and time consuming data manipulation.

MS detection is generally performed in multiple reaction monitoring (MRM) mode, giving the maximum specificity and sensitivity for each compound. The MRM methods are developed automatically using QuanOptimize. This software automatically selects the mode of ionisation, precursor ion peak, the optimal cone voltage, fragment ion, and most favorable collision energy. This can be achieved under complete automation requiring only the input of the molecular formula of the compounds upon submission.

Commensurate with the need for high speed data acquisition is the need to rapidly process the data. This requires peak detection, integration, quantification, and reporting. This can often be a very time consuming activity requiring the laborious transfer of data from one software package to another. The use of ProfileLynx eliminates the need for this data transfer as well as any transcription errors associated with the transfer. Thus all of the sample queue generation MS method development and data analysis can be carried out with one software solution.

As the volume and complexity of research data continues to grow, the use of an electronic format for laboratory record keeping is the key to simple recording and global communication of critical scientific information. This medium offers benefits over traditional paper-based notebooks as it requires less physical storage space, provides faster and easier search capabilities, and captures graphic and text-based information easily without transcription error. Thus, experimental details can be recorded in an efficient, legible manner to create a shareable and searchable environment for present and future use.

As the networking of laboratories becomes commonplace in many organizations, researchers require new means to organize and comprehend results. Keeping pace with advances in the Waters Informatics portfolio of solutions, Waters has developed SDMS Vision Publisher to seamlessly interact with existing data products for streamlined usability and maximum productivity in a secure environment.

![Figure 5. The ADME workflow.](image-url)