

# ***Integration for Medium to High Throughput HPLC Purification***

By Rob Darrington, Product Manager, Genevac Ltd.,  
Paul de Fraigne, Group Leader, Chem Tech Group Syngenta and  
Mike Jones, Group Leader, Scynexis Europe Ltd.

## **Introduction**

In the modern purification laboratory, many thousands of novel drug-like compounds are being purified each week. Preparative high performance liquid chromatography (HPLC) is the method of choice. A major issue within the laboratory is traceability of each compound, tube, and fraction. What are the costs of confusion in the laboratory? Lost sample, lost time, lost money, lost contract, lost job?

Typically, a 5 to 10mg injection of compound will produce anywhere between 2 and 5 fractions, depending upon various factors, including; initial purity of sample, method of triggering fraction collector, pump speed and fraction collection tube volume. This begins to create a sizeable problem. Even in a lower throughput lab, this is clearly a huge increase in number of tubes to track. Downstream of HPLC samples need to be analysed, often a daughter sample is taken for analysis which is another doubling in sample numbers. The problem has become much worse! The remainder of the process is usually simple, drying, redissolving, and combining to obtain one final sample of pure compound, usually in a new tube, vial, or plate well. What are the options for managing traceability? This article looks at some of the options and studies the processes of two leading companies who have developed successful solutions.

## **Number Crunching**

As we have seen, 500 samples entering a lab can easily become 1,000 fractions, 1,000 daughter samples for analysis, and a further 500 pure samples in a final format for storage or shipping. The surest way of tracking each sample and fraction is to barcode label each and every tube, however, this is laborious and time consuming. Additionally, this generates a huge array of data to store and search, in order to find and sort any given sample at the end of the process. That said, this is not too difficult to manage, with suitable data collection systems, although it is time and labour intensive, when other solutions are available. What is clear, is that whatever the solution, a good data tracking software package which integrates with existing systems would be a sound investment. Even so, care is needed when manual handling is done, there are around six or seven manual manipulations required between stages throughout purification and associated processes, and each may lead to error. Even though the tube is labelled, it still must be in a known, recorded location.

## **It's all in the Technique**

While it is well known that mass directed preparative HPLC generally yields a lower number of fractions per injection than ultraviolet (UV) or evaporative light scattering (ELS) directed HPLC, it is much slower, which compromises throughput. While UV or ELS direction is faster, more samples are collected which adds to the work load of drying, combining, checking and tracking fractions. Is there a sensible compromise? In an ideal world, routine separation would probably be done using UV directed chromatography and the more difficult separations with higher levels of impurity or close peaks, would be routed via mass directed systems. However, this is too simplistic. An option would be, to save time by using a robot system to do the sample handling following UV directed HPLC, which on balance shows a preference for this route.

However, once the cost of a capable robot and tracking software are added to the price of the system, it may begin to approach the cost of a mass directed system, with only a few hours in processing time being the key difference. A time and motion study would be needed to assess each individual laboratory's needs against these two possible scenarios.

## Whole Rack Solutions

Another solution to this problem, is to use sample racks as a carrier throughout the process. This is less labour intensive, because the rack only needs to be barcoded (usually a one off operation), and loaded with clean tubes. Tubes may be weighed by robot, if required. The identifier for each tube is its position within the uniquely labelled rack. Can this really work? One laboratory Head commented that, "suppliers need to have their heads banged together, and decide on one common format, that fits all system requirements."

There are some issues which are clearly difficult to resolve. However, given that these can be overcome there are benefits to this approach. From a manual handling point of view, if the rack can travel through the system as the common carrier, then manual handling of individual tubes is eliminated, save for loading empty racks and unloading used full racks. The operator handles just the rack between operations. This will significantly reduce tracking errors, and help prevent accidental loss or damage. Standard racks can usually be designed to integrate between systems, for example, weighing robot, HPLC fraction bed, and liquid handler. In some cases modifications are required to the sample holding bed of some systems, although this is usually a trivial cost when compared to the costs of confusion. An issue does arise when racks reach the drying step. Centrifugal evaporation is commonly used to dry fractions, being the fastest safe method available. The problem occurs in the evaporator, where standard tube racks would collapse under the g-forces of the centrifuge, and, the evaporation performance in a standard rack is poor due to their low thermal mass.

## Drying Dilemmas

To circumnavigate the drying problem where racks do not fit into a centrifugal evaporator, a simple solution would be to use a different solvent removal method. This is possible, but has a time, and in some cases a purity penalty attached. Solvent bumping in freeze driers is a problem where fractions contain organic solvent and water, and few things elute in 100% water! To make this work, it would require another technique to remove the organic fraction, for example, nitrogen blow down, which introduces more expense and user intervention. Blow down by itself would be very slow due to the high latent heat of vaporisation of water, requires large volumes of inert gas, and, has a greater environmental impact with significant volumes of organic solvent vapour to handle. On the plus side, freeze dried samples are weighable and can be very easily redissolved.

The obvious solution to this is to use racks which can be used throughout the whole process, and not just part of it, including the evaporator. To be suitable for this purpose, the racks must integrate with the weighing robot, fraction bed, and liquid handlers, but also be strong enough to withstand high g-forces and have good heat transfer properties. Genevac has several solutions to this problem.

For users of Gilson 200 series and other large format racks, Genevac have a range of look-alike racks which integrate with the other systems in the process, and are suitable for the evaporator. These racks not only withstand the g-forces involved but have a much shorter drying time, typically one quarter that of a traditional rack.

[See <http://www.genevac.co.uk/presentations/comparison/index.html> for details].

This is a good solution for those with very high throughput and space to play with, although for many space is at a premium, and systems which can accept these big racks have, necessarily, to be large.

For users of these large racks, a different approach to solve the same problem, is to have racks which break down into smaller parts. Each sub rack is uniquely labelled, and can fit into a smaller evaporator. Genevac can provide two solutions. For larger tube sizes, e.g. for 30ml fractions, the large rack holds a number of tube holders which can be placed directly into the evaporator. For smaller tube sizes, the rack is made up of microtitre footprint sized holders, which fit into standard evaporator swings. These work as well as the whole rack approach, and have the benefit of maintaining a continuous flow of work through the laboratory.

Using a smaller evaporator, means that it is easier to fill, and keeps fractions moving, rather than waiting for a number of large racks to fill, before starting the evaporator. Evaporation times are faster in smaller systems, because there is a lower volume of solvent to remove. The final stage in this thinking, is to use microtitre sized holders without the large format 'mother' rack. These can be manufactured to integrate with most fraction beds, without modification to the bed.

A potential disadvantage to drying the whole rack is that often not all tubes on the fraction bed need to be dried. Either the contents of a tube is not required, or a sample elutes in a small volume of solvent, and leaves a half empty tube. Hence for some configurations of HPLC system it may be more suitable to cherry pick and individually label tubes for drying. One alternative is to accept some redundancy in the system and dry even unwanted samples. A different solution is to have a process which helps, for example using simple automation coupled to good sample management software it is simple enough to create a daughter plate of samples, which when analysed show which samples are wanted. The desired samples can be cherry picked and assembled into suitable sample holders for drying. If cherry picking is automated, the sample holder can be suitably labelled and become the new carrier.

Another other key issue is balancing the centrifuge. No two fractions are alike in terms of solvent ratios (hence drying time) or volume. Centrifugal evaporators which may start the run balanced, can quickly become imbalanced as the organic solvent evaporates. There are two solutions to this, program the fraction dispense head to fill racks in such a way as to avoid getting all water and all organic in one holder, and/or, buy a better evaporation system, typically one that has high imbalance tolerance and an autobalancer fitted.

A further consideration is does the evaporator rapidly dry each and every sample fully? It is not unusual when drying preparative HPLC fractions to find that random samples do not fully dry due to the formation of an azeotrope when the compound in the water reaches a certain concentration. If such a situation arises, what are the options? If the whole rack is put back in the evaporator, what may happen to the already dry samples? Sublimation of dried compound, and heat damage are certainly potential risks. Alternatively, the wet samples can be cherry picked out and dried separately, however, this again raises the issue of traceability. Using the very latest evaporation technology, these samples can be rapidly dried without damaging neighbouring dry samples by carefully controlling the evaporation process at all stages. Genevac provides free applications support to all users for the lifetime of the system to help users achieve this.

### **The Syngenta Solution**

The combinatorial group at Syngenta can purify up to four hundred samples per week automatically by mass directed HPLC. This is not a huge throughput in comparison to some laboratories, however they have an interesting and well integrated process, as shown in the flow diagram. The process is designed to take in a crude plate, and return a purified plate for follow up biological screening.

During the integrated process there are liquid handling and evaporation steps, with the samples travelling between the Genevac, Bohdan USP, Waters FractionLynx and Hydra either in microplate or collection rack format. If each sample tube were labelled traceability would be a serious problem (unless an individual barcode on each tube were used). Integration of the whole process means that it is very flexible to cope with various sample formats and amount so that it is possible to pick and choose parts of the operation.

For the front and back part of the process Syngenta use microtitre plates, either in fixed well or Micronics tube rack format, where sample tracking is relatively simple by plate and well location reference. For fraction collection, microtitre sized solid aluminium sample holders loaded with 24 glass 16x100mm tubes (see picture) are used. These holders fit onto the Waters fraction collection bed, and are also suitable for use in the Genevac evaporator and on the Bodhan USP. Each rack is marked with a unique identifier to allow it to be tracked through the system. It is simple enough to track these sample holders as they are always used in sequence. The fraction tubes are tracked by this sample holder number and it's position within the array by the software.

Without the use of these holders there would have to be an increased input from the operator, with the tortuous process of transferring individual tubes to three different racks, one for the Genevac evaporator, the fraction collector and the Bodhan USP. By using these evaporator sample holders to ease workflow through the system, sample misidentification and manual handling are both significantly reduced.

## **The Scynexis Solution**

Scynexis run a high throughput compound synthesis production line, and therefore require a very high throughput purification system. Typically they will have in the order of three thousand crude compounds per week, to purify. Purification is achieved using their own proprietary work up and HPLC method. Purification generates an average of two 8 to 10ml methanol water fractions per injection.

The fraction collection systems used are Gilson 215 units, however, the tube racks are not a standard Gilson 207 rack. The racks used are a Genevac designed look-a-like rack built to withstand use in a centrifugal evaporator. Racks have a deep solid aluminium base to provide excellent heat transfer, and a robust top plate to support the tops of the tubes. These racks mimic the location and other features of standard Gilson racks in every way. Each rack is bar coded to ensure efficient tracking.

The racks are loaded with tubes and placed on to a Bodhan USP. The tubes are tare weighed, and the racks go into the bank. Tared racks are loaded onto the fraction collection beds, scanned with a bar code reader and rack position is logged in the database. Fractions are collected into the racks. If a rack is not totally filled with fractions from one plate of compounds, it stays on the fraction collector until fractions from the next plate of compounds use up the remaining tubes. Scynexis have a very efficient database which tracks and data logs each sample and rack throughout the whole process, eliminating human errors. Full Gilson racks are loaded into the Genevac Mega 1200 evaporator and dried, taking approximately 6 hours to achieve total dryness. Following drying, the tubes are reweighed on the Bodhan, and then are transferred to a Tecan liquid handler for addition of solvent to provide a wet, pure sample of known concentration. Samples are now ready for QA and subsequent reformatting into the final desired format.

Human intervention in this process is limited to moving the racks between machines, and scanning the barcode of the rack. The database logs the relevant data, and even directs the liquid handler as to how much solvent to add to each tube. The keys to the system are the Gilson racks suitable for all steps of the process, and the data management system.

## **Summary**

Tracking sample tubes through a purification laboratory can be a torturous process. Traceability can be improved by the use of sample holders which integrate with the systems used at each stage, coupled with suitable sample tracking software. There are a number of potential difficulties which must be overcome to make this system work, particularly in the area of evaporation. By using the latest technology available from the quality supplier, this can be achieved.