COMPOUND MANAGEMENT

in pursuit of sample integrity

In December 2004 HTStec published its report on Compound Management Trends. This market report summarises the results of a global benchmarking study on pharmaceutical compound management (CM), documenting current practices, issues and future trends in CM, with a particular focus on compound quality control. This article reviews some of the survey's main findings and provides an overview of the current status of liquid compound storage across the industry. It also takes a detailed look at the main issue (sample integrity) and the biggest bottleneck (compound retrieval and adequate cherry picking capacity) that CM groups reported. The survey findings are used as a backdrop against which the some of the latest technologies now being applied to compound management are discussed and new developments in tube-based storage systems are reviewed.

By Dr John Comley

In December 2004 HTStec undertook a global benchmarking study on pharmaceutical compound management (CM). The objective of the study was to comprehensively document current practices, issues and future trends in CM, with a particular focus on compound quality control and sample integrity.

Current practice in liquid compound storage

Based on feedback received in this study the mean size of a compound library today (2005) in the large pharma is 1.05 million compounds, this is expect to grow 1.80 million by 2008. The mean size of a compound library today (2005) in the small pharma is 0.73 million compounds, this is expect to grow 1.25 million by 2008 (**Figure 1**). On average around 90% of these compounds are accessible for screening. The study also revealed that about 60% of all screening operations have access to some form of automated liquid CM store. The remainder still use an ad hoc arrangement of lab freezers as the main component of their CM store. The majority (55%) use microplates and microtubes for storage in part of the same CM facility. Only 20% use microplates alone and a further 25% use only micro tubes. Nearly all (89%) storage facilities use 100% DMSO (assumed starting concentration) for their stock solutions: of the remainder 6% use 90% DMSO, 2% use 75% DMSO and 4% do not use DMSO.

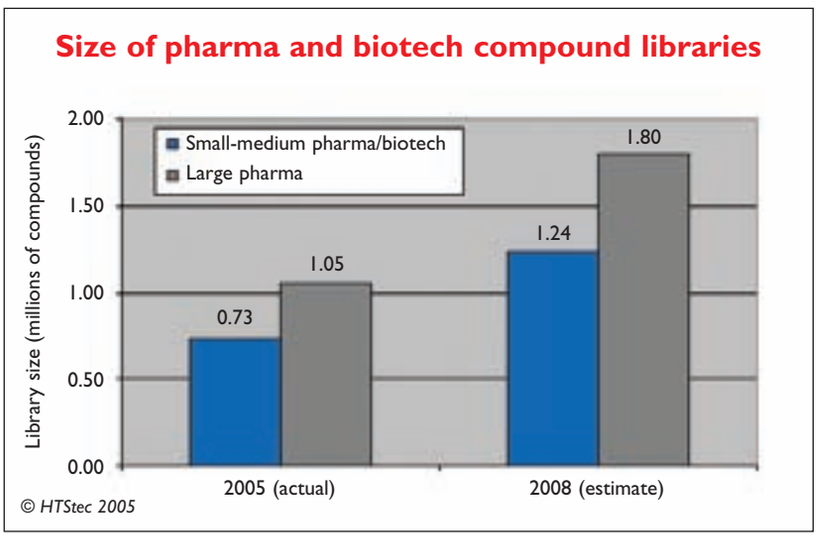


Figure 1

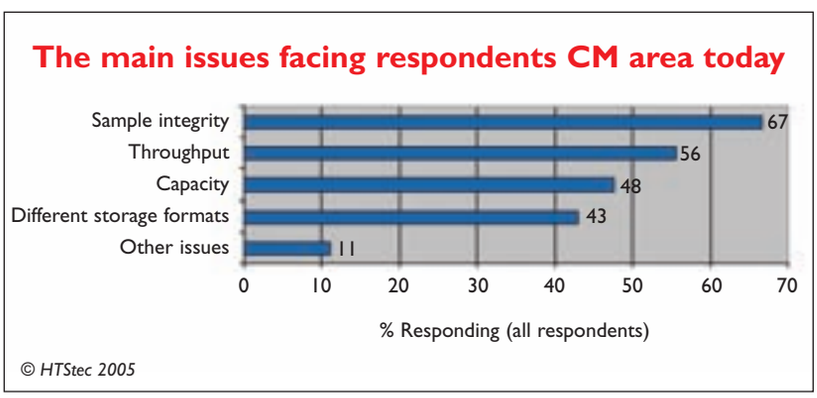


Figure 2

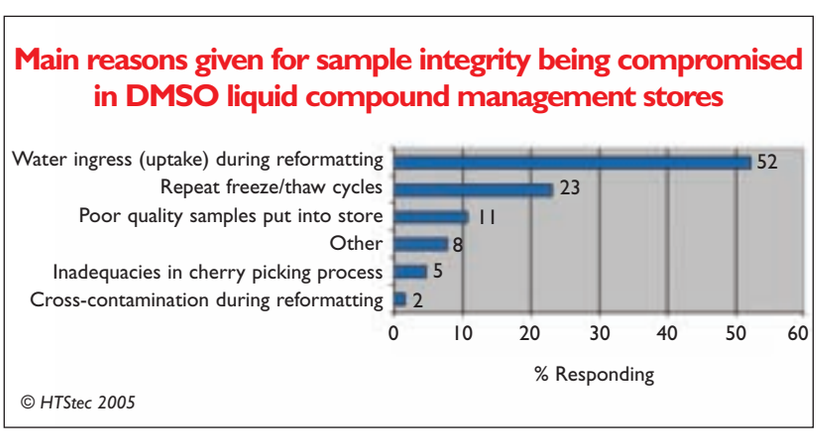


Figure 3

Sample integrity

Pharmaceutical screening operations today are increasingly focusing on achieving and maintaining quality. In compound management groups this is reflected by their desire to ensure sample integrity, both with respect to new compounds entering the collection, and the handling and storage of existing compounds. The importance compound management groups currently place on sample integrity was evident in the HTStec recent survey (**Figure 2**). Sample integrity is generally regarded as a measure of the quality of the material in a container (eg vial, tube, microplate well, etc) and relates to: 1) the degree to which the chemical identity (structure) of a compound in a collection is what it is supposed to be. Quite frequently the starting material used to prepare the solution is not what is on the label; 2) the purity of that sample, are there any impurities or salts present; these may arise for example as a consequence of impurities introduced (or not eliminated) during original synthesis or new species arising in consequence of compound decomposition; and 3) the actual concentration of a liquid sample in DMSO, how does it deviate from the intended concentration at the time of solubilisation, and also includes any post solubilisation dilution processes. Here the influence of precipitation, water uptake and solvent evaporation are all significant. Making sure that compounds received into a library at the start are in fact of high purity, as well as being the compound they are supposed to be is therefore highly important.

HTStec's survey of CM groups revealed that water uptake (ingress) during the reformatting process was seen as the main reason for sample integrity being compromised in DMSO liquid stores, ie during the transfer of aliquots of compound from a library stock into an assay plate (**Figure 3**). Repeat freeze/thaw cycles were ranked the second main cause for loss of sample integrity. Cross-contamination between wells can also be regarded to compromise compound integrity. Implementation of quality control (QC) measures to assess sample integrity has rapidly become a major priority for compound management groups.

QC measures

HTStec's survey documented some of the QC measures that either have been applied to compound collections or are being considered for the future (**Figure 4**). Of those applied to the entire compound library, regular QC of liquid handing equipment and volume measurement (tracking volume remaining) have been implemented by most companies. Of the QC measures being applied to pre-selected and randomly selected compound, integrity/structural validation and purity have been implemented to the greatest extent. HPLC separation followed by mass spec (LCMS) detection is widely used in QC analysis to determine concentration (and purity).

The survey also asked respondents about the

Figure 4

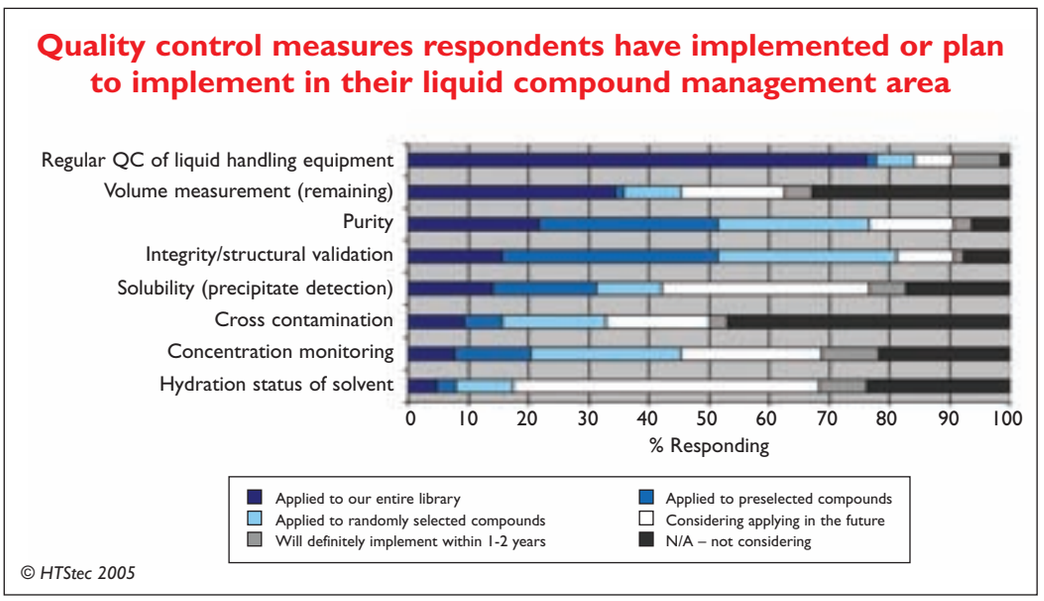
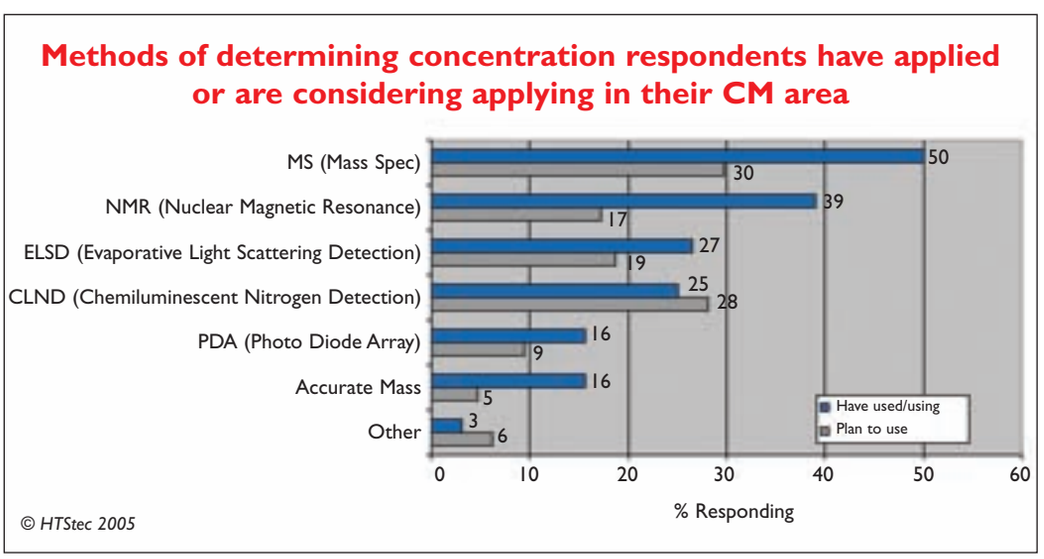


Figure 5



methods they had applied or are considering applying for concentration determination in their CM area (**Figure 5**). The results confirmed the recent industry focus on the use of the evaporative light scattering detector (ELSD) and the Chemiluminescent Nitrogen Detector (CLND), as part of a drive towards obtaining a universal detection scheme to improve single calibrant quantification[1]. Previously compound analysts have relied on UV and MS to ensure all components are detected. However, compounds without a UV chromophore, or those that do not ionise easily, will be missed by both these detectors. Consequently, the evaporative light scattering detector (ELSD) has proved a useful tool in this field as it responds to all compounds that are less volatile than the mobile phase. ELSD can also handle high flow rates and rapid gradient separations that are typically employed in higher throughput methods. ELSD is a three-stage process, ie nebulisation, evaporation of the eluent and optical (UV) detection. Direct injection of DMSO solutions for HPLC analysis is impractical when using a UV detector because the signal from the DMSO peak may mask some early eluting compounds. However, with a **Polymer Laboratories** (www.polymerlabs.com) ELSD (PL-ELS 2100) the DMSO signal can be eliminated without increasing the temperature by the addition of a carefully controlled stream of gas to the evaporation step thereby enabling any early eluting

Polymer Laboratories' evaporative light scattering detector – PL-ELS 2100

semi-volatile analyte to be detected. In addition, significant cause of library degradation is when compounds are stored as trifluoroacetate (TFA) salts in solutions of DMSO. These TFA salts are formed either as a result of cleavage of the compound from a solid support, or from final stage preparative HPLC purification where the solvent system employed typically contains a small percentage of TFA by volume. The generation of free-base compounds prior to storage is therefore thought to be an effective way of preserving library lifetimes. By using a simple sample preparation technique utilising an appropriate polymer support (eg Polymer Laboratories' StratoSpheres SPE (solid phase extraction) product PL-HCO3 MP SPE), compounds can be easily converted to the free base form prior to archiving.

While LC-UV-ELSD-MS can be used to confirm the purity and identity of compounds archived in screening collection, it is desirable to monitor the true compound concentration in DMSO as this will impact on screening results. One way of monitoring the concentration of compounds is to use CLND. Methods based on traditional detectors, such as UV, can be misleading and time consuming because detector response is dependent on chromophores requiring purified references for every single compound of interest. **Antek**'s (www.antekhou.com) Model 8060 CLND is a nitrogen specific HPLC detector that uses a single, high purity nitrogen calibration standard, although the calibration need only be run once daily. The 8060 is based on Antek's Pyro-chemiluminescent™ technology, and delivers an equimolar response for all nitrogen containing compounds. Chemiluminescence is the clean, fast, interference-free method for determining all bound nitrogen in a variety of matrices. The chemiluminescence reaction is as follows:

$$R\text{-}N + O_2 \rightarrow 1050°C \rightarrow NO + \text{combustion products}$$

$$NO + O_3 \rightarrow NO_2^* + O_2 \rightarrow NO_2 + O_2 + \text{light}$$

Recent pharma advances suggest that the CLND may be a better detector than ELSD and its use has been validated for monitoring the purity and concentration of DMSO samples. However, ELSD may have a role as a back up for non-nitrogen containing compounds and to diode array as it is good for detecting compounds with no chromophore[2].

It is also possible to quantify small amounts of material using NMR by integrating the total proton spectrum, and the survey indicates the use of NMR is widespread (**Figure 5**). Use of an added eretic reference signal allows accurate and precise quantification by NMR without the use of HPLC. Throughputs achieved today with autosamplers are roughly comparable to CLND, the question is does this make effective use of an expensive tool like NMR, which might be more usefully deployed for structural elucidation. A further issue with NMR is the necessity for (human) spectra interpretation.

Current methodologies like CLND and NMR although accurate and sensitive enough are not really of the throughput desired by pharma for concentration testing. They are adequate for a one off assessment, but they could not routinely be applied, to check if the concentration had changed during storage, to liquid samples in corporate compound collections at the point of delivery to the customer (ie measure the actual sample concentration of the sample diluted in the assay buffer in parallel with the screen) where the number of analyses required would be considerably larger.

Acoustic auditing

Of the QC measures being considered for the future documented in HTStec's survey, greatest interest was shown in the hydration status of the DMSO solvent and solubility (precipitate) detection (**Figure 4**). Survey respondents considered acoustic auditing, as exemplified by Labcyte's Echo 550 compound reformatter, as the most promising new tool for the quality control of compound libraries. They deemed it superior to

other techniques for its ability to determine, quickly and non-invasively, water concentration in DMSO stocks.

At the forthcoming SBS Meeting in Geneva (September 11, 2005), **Labcyte** (www.labcyte.com) will commercially launch the Echo™ 380 Auditor. The Echo Auditor adds value to compound management operations by monitoring both library source plates and drug candidate assay plates. In the former, the 'health' of the compound library can be measured by detecting the DMSO hydration status, checking for low wells due to either evaporation or having been 'cherry-picked' to dryness. This prevents researchers from measuring the activity of null transfers. In the case of assay plate QC, the Auditor monitors the quality of bulk reagent dispensers and pipettors and can quickly inform the researcher if dispensing lines are blocked or if volumes dispensed are anomalous. The Echo 380 Auditor audits both microplates and microtubes. The Echo Auditor is compatible with an increasing number of plates and microtubes (consult Labcyte for latest details). Labcyte anticipates warranting well volume and DMSO hydration with both accuracy and precision between 3% and 10% depending on the composition and geometry of the container (eg V-bottoms are most challenging, flat is most accurate; thin bottomed plates are more challenging than thicker bottoms). The range in accuracy and precision is largely driven by the uncertainty in measuring the speed of sound in the liquid.

Figure 6 shows how acoustic auditing, as offered in Labcyte's Echo 380 can be used to 'listen' to a well. A transducer is positioned below a well or microtube. A layer of fluid (water) transfers acoustic energy between the transducer and the container. Focused ultrasound generated by the transducer propagates into the coupling fluid and some of this energy reflects at each interface encountered by the beam. The impedances of the various materials are shown. Water has an impedance of approximately 1.5 MRayl. The plastic wells have impedances around 2.5 MRayl. The fluid above is often an unknown mixture of water/DMSO, and the impedance range for these binary mixtures spans 1.6 to 1.85 MRayl for the range of anhydrous DMSO (100%) to laboratory atmosphere equilibrated DMSO (as low as 60% DMSO:40% water by volume). The amplitude of the second echo (reflection outlined in grey) is key in determining the impedance of the fluid in the container. If the amplitude of this reflection is known, the impedance can be determined, and hence the DMSO/water mixture calculated. The uppermost reflection is at the fluid/air interface,

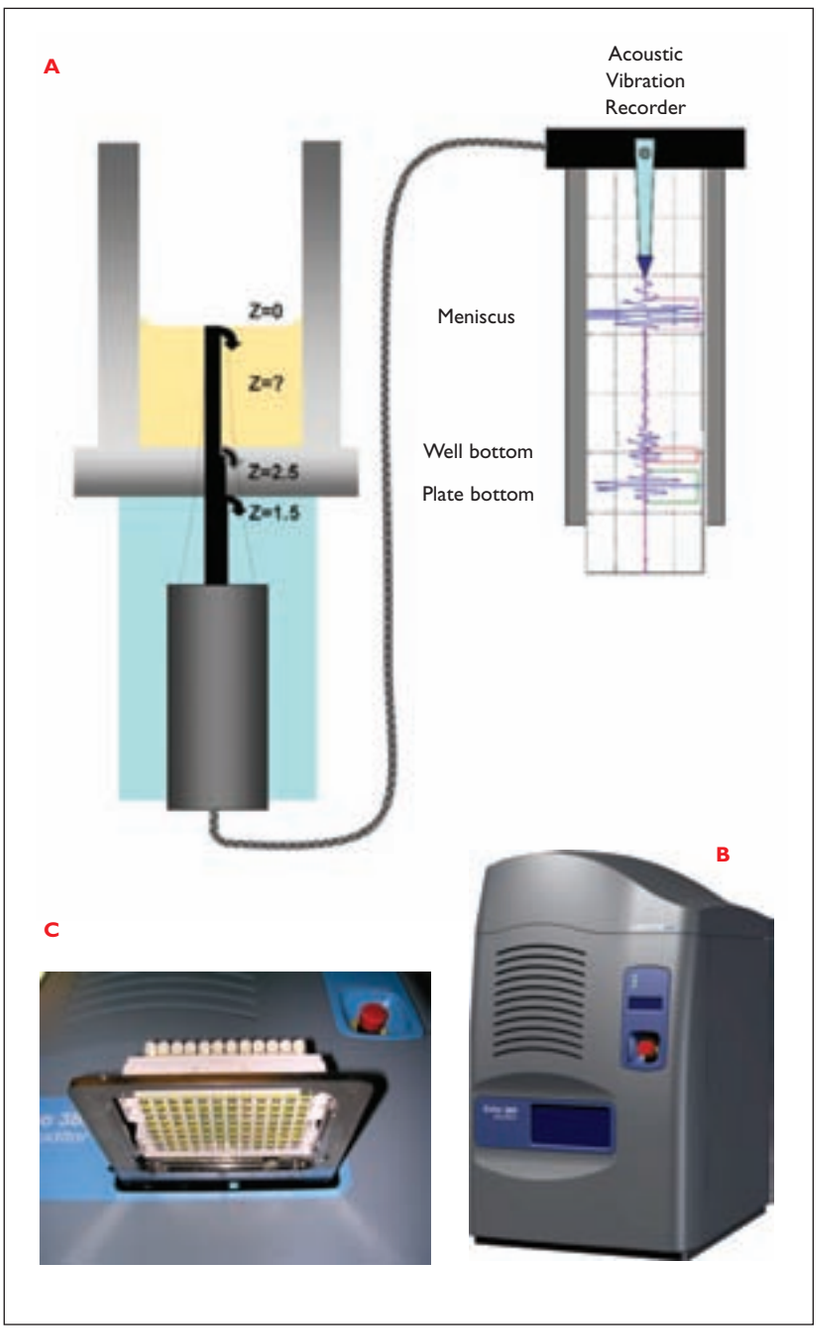


Figure 6
A How acoustic auditing using the Labcyte's Echo 380 Auditor can be used to 'listen' to a well or microtube. **B** Labcyte's new Echo™ 380 Auditor can measure the water concentration in DMSO stocks and the volume of liquid in wells or microtubes. **C** A plate of 96 storage tubes being loaded into Labcyte's Echo™ 380 Auditor

where essentially all the acoustic energy is reflected back into the liquid. Each of these reflections travels back to the transducer. After launching the initial acoustic energy pulse, the transducer is switched from being a generator to being a receiver. This is shown in the diagram as a cable connecting the transducer to a vibration recorder that operates like a chart recorder for monitoring seismic pulses of earthquakes. Note that each of the reflections arrives at different times, and they have been illustrated here to align with the location of the physical feature (meniscus, well bottom and plate bottom). They have different amplitudes as well, the most important being the amplitude (the left-to-right excursion of the pen of the chart recorder) of the well bottom as this enables the determination of the impedance of the fluid in the well. The determined impedance is converted to a unique value of DMSO hydration and a unique speed of sound. The time delay between the meniscus echo and the well-bottom echo is converted to fluid depth with the knowledge of the speed of sound in that particular well or tube.

Compound precipitation from DMSO stock solutions is increasingly viewed as a more serious problem than chemical degradation. Current opinion is that uncontrolled water uptake into DMSO stocks in synergy with freeze/thaw cycles is the primary cause of precipitation[3]. Respondents to HTStec's survey were asked for their opinion on the best approach to deal with compound precipitation, the results are presented in **Figure 7**. Opinion was divided between those who advocate trying to prevent the problem in the first place, ie the use of single shot/single freeze thaw tube approach under controlled environment in the compound storage area; and those who accept the problem exists in their collection and place more emphasis on measuring solubility when the compound hits the assay. Of the other approaches the use sonication device to resolubilise compounds is perhaps the most amenable to implementation.

Sonication

Sonication (the application of high-frequency acoustic energy to a sample via a transducer) at frequencies near and beyond the upper limit of human hearing give rises to non-linear processes such as cavitation. These effects are responsible for the utility of sonication in mixing and solubilisation. **MatriCal**'s (www.matrical.com) SonicMan™ is a unique highly parallel version of a traditional single probe sonicator, in which disposable metal probes (pinned plate lids) are immersed into sample wells to directly apply sonic energy. It provides simultaneous contact sonication of all wells of a 48, 96, 384, 1536 well, or custom formatted plate, allowing for orders of magnitude increases in throughput relative to a single probe sonication horn, without adversely effecting chemical stability and without significant heating.

An unknown but significant fraction of compounds dissolved in DMSO may not go into solution, or may go into solution only to precipitate out at a later time as a result of water pick-up from

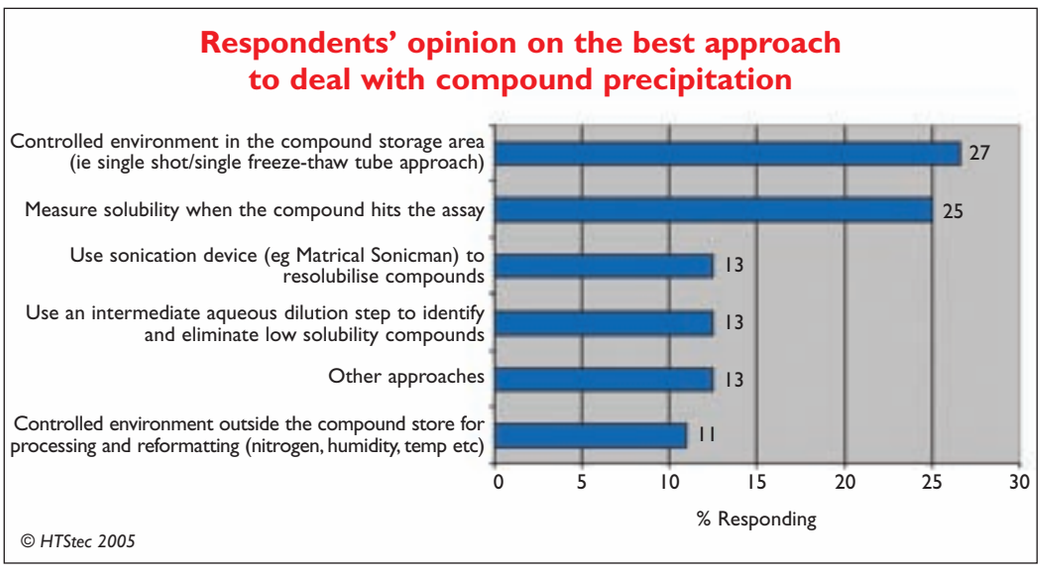


Figure 7

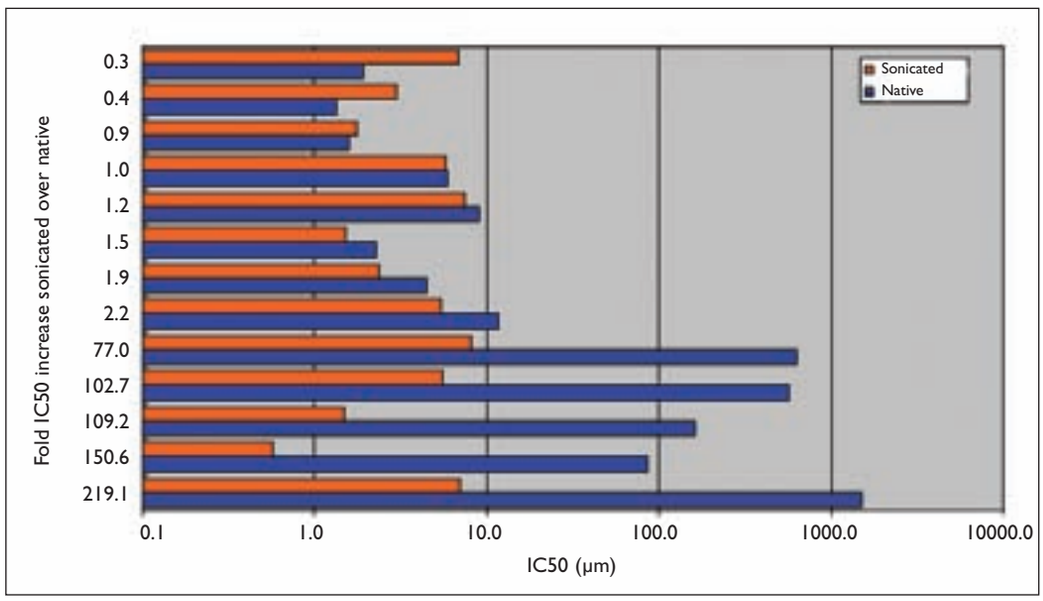
multiple freeze-thaw cycles. It has been estimated that 40% or more of compounds stored in compound management systems throughout pharmaceutical companies are precipitated upon freeze thaw. Once compounds precipitate from wet DMSO, redissolution is very difficult and simple methods are not very effective. Shaker table mixing, the traditional solubilisation technique, may require from several hours to overnight mixing to get stubborn compounds to go into solution. By sonicating those samples with SonicMan™ just prior to liquid handling, those compounds which are capable of being dissolved will be driven back into solution, at least to the limit of their thermodynamic solubility. This can be accomplished in seconds, as opposed to hours for shaker table solubilisation. Resolubilisation just prior to screening eliminates one major source of false negatives. It can have a noticeable impact on the quality of hits by facilitating better, more consistent data for structure-based screening data analysis software and SAR models (**Figure** 8).

Plate-based versus tube-based liquid compound storage

The big debate in CM as to whether plates or tubes offer greater flexibility and minimal adverse effect on sample integrity for liquid DMSO stocks, has

Far left: Matrical's individual matritube gripper picking a 96 well 2D MatriTube
Left: Matrical's SonicMan™

Figure 8
Shows the IC50 results for 13 compounds which were hits in a primary screen. The IC50 determinations were made both with and without sonication of the stock DMSO solution of the compound prior to aliquotting for the concentration-response experiment. Five of the 13, or almost 40%, showed a roughly two order of magnitude improvement in the measured IC50 as a result of sonication with Matrical's SonicMan™

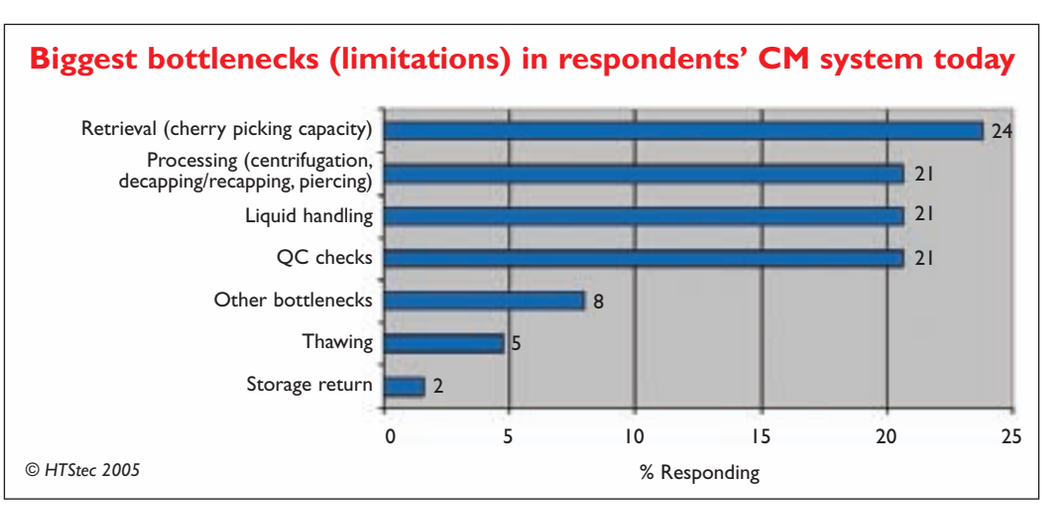


largely been won by tubes, if current vendor promotion of tube-based systems is an indication. However, most Pharma today make use of a mix of both storage containers for their liquid library.

Plate-based storage provides samples prearrayed in the plate format of choice, typically 96 or 384, but today there is increasing interest in 1536 well plate storage. Samples are frequently sorted into sub-sets represented by a collection of plates. Plate storage particularly facilitates the preparation of replicate copies of those stocks, using a multichannel pipettor, and the shipping of liquid stocks to different labs/satellite stores. However, plates need to be delidded or desealed or matts removed to gain access to the samples contained in the wells, in many cases this is a manual process. Although it is technically possible to pierce individual wells, in practice this is not common and removal of one sample from a plate typically involves exposing all samples to the operating environment. In addition, it is currently not possible to freeze/thaw individual wells without thawing the entire plate. Furthermore, cherry picking can be time-constrained if the samples of interest are spread over a large number of different plates. Cherry picking involves removal of an aliquot from a well, and picked plates may end up with some wells devoid of compound, while others are untouched. Although plate storage has been successfully automated, it is also readily amenable to manual processing, and as such is widely used by groups (eg small biotechs and academic labs) which have not invested in automated compound storage and retrieval systems.

Tube-based storage is associated with a variety of

Figure 9



formats. Tubes of various sizes (volumes) may be stored in racks, in racks (or inserts) on store trays and direct in special store trays. Minitubes are generally stored in special receiving microplates (96 and 384 well formats only). Racks and store trays may take the form of a microplate array or unique close packing array (eg TTP Labtech comPOUND). Tubes are usually picked into a microplate format so they can be processed (eg diluted) or replicated en masse like a microplate. Most tubes are identified by a bar-code, eg a 2D code on the tube base. Some types of tubes can be sealed with either a pierce-cable septa or a removal cap, facilitating access multiple times to the tube. Alternatively, DMSO resistant seals (eg plastic coated aluminium foil) are applied that can be pierced only once. Usually these seals are applied to an entire plate of tubes and are then cut into individual (singlicate) tubes 96 or 384 at a time. The main advantage of tube-storage is the ability to pick a single tube from within a controlled dry environment without compromising the integrity of the other tubes. The trend today is towards single shot (use) and single freeze-thaw of foil-sealed minitubes that contain a minimal stored volume (<25µL) of DMSO stock solution. Reasonable fast random access cherry picking can be accomplished by XYZ robots that physically pick (remove) tubes with a gripper from a storage tray and place them into a receiving tray or plate (eg TekCel, Matrical). Some minitubes are pushed upwards out of the source plate into a gripper that can hold multiple (12) tubes (eg TAP), which reduces travel and gives higher pick speeds. The picking tolerances of 384 mini-tubes are, however, tiny so for higher speed and accuracy some companies advocate avoiding XYZ robot manipulation. Such mini-tubes maybe picked by punching out tubes, arrayed in the microplate, through the base into a receiving plate positioned below (eg REMP). Faster picking rates are achieved if this can be done en masse or in parallel (eg RTS Life Science), rather than punched singly. The pick rate of comPOUND (TTP Labtech) which uses pneumatic picking to is 5sec/tube irrespective of the tube location in the store, however with multiple comPOUND stores you could get up to 12 tubes in arrive in parallel

every 5 seconds (ie 0.4sec/picked tube). The latest tube stores are designed to enable high speed picking, with advertised pick rates of between 1,000-5,000 tubes/hour. Fast picking is particularly desirable for 'smart screening' of smaller, focused iterative sets, but also for the initial hit validation and IC50 phase. The importance of adequate cherry picking capacity was underscored by the fact that survey respondents ranked compound retrieval as their biggest bottleneck (limitation) in compound management today (**Figure 9**). The cherry picking rates achieved by end users with real libraries today and those respondents' desire for various aspects of drug discovery are somewhat at variance (**Figure 10**). Furthermore, is can be somewhat misleading to assume that the picking rates quoted by vendors capture the reality once a system is installed.

What determines retrieval rates in automated compound management?

The effective picking rate of an automated compound store does not only depend on the cycle time of the picking robots, but is typically limited by a range of factors including the order size distribution (many small orders are typically much less efficient than one large order), whether samples are retrieved as entire racks or individual cherry-picked items, the number of items on a storage tray etc relative to the time taken to fetch it, whether required items can be co-located on the basis of likely retrieval patterns, whether alternative vessels are available which can be selected dynamically to maximise efficiency, whether sample identity is verified during picking and whether this represents a time overhead on the picking process, and, especially at higher rates of access, how efficiently the store logic can deal with loading samples back into the store at the same time as needing to service retrieval orders.

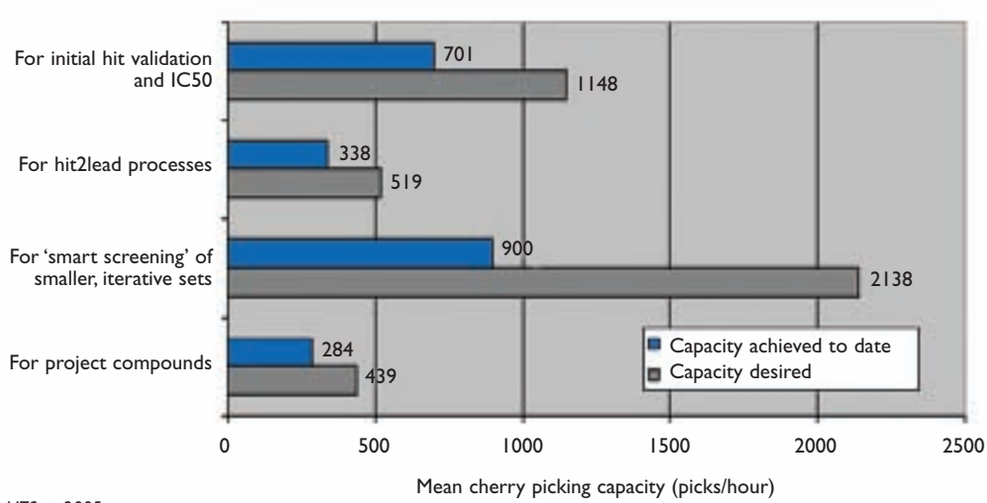
Unless careful attention is paid to all these factors, the performance of an automated system may be disappointing compared to what might be theoretically possible based on the robot cycle times, and throwing more hardware at the problem may not be the answer.

The following are some of the latest developments in tube-based compound storage systems from vendors active in this increasingly competitive market place.

REMP (www.remp.com) led the way to a new quality standard for sample storage when it developed its pioneering 384 Tube Technology™ in 1997. The introduction of individually sealed tubes and the concept of storing small aliquots ready for single use eliminated unnecessary freeze/thaw cycles, carry-over and dilution effects as well as greatly reduced water uptake and exposure to air, which is intrinsic to traditional sample preparation and liquid/solid transfer steps. The simple and extremely reliable tube punching mechanism, which is the core of the REMP Tube Technology™, also led to a dramatic decrease in the error rates for tube picking as compared to traditional pick and place methods. In addition, to

Figure 10

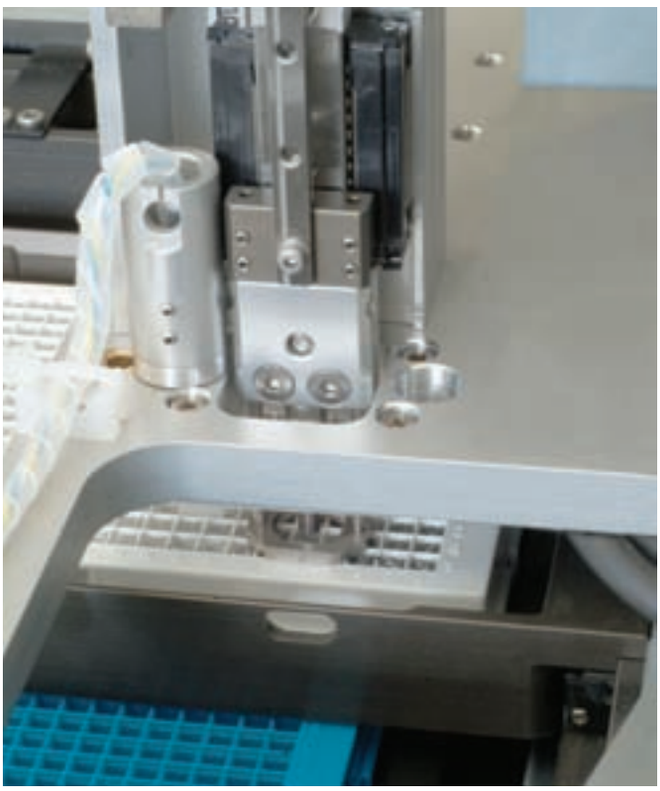
Cherry picking capacity – what has been routinely achieved and what is now desired by respondents



its larger Automated Sample Store (ASS), REMP now offers a new series of Mid Size Stores (MSS) with a capacity range from 20,000 to 78,000 shallow well plates depending on the configuration. Offering standardised sample storage systems has led to a dramatic decrease in time from initial order to onsite operation. Within REMP's stores tubes are manipulated in a unique 'punching' fashion through the use of an automated device, the Tube Punching Module (TPM). All tube transfers are logged in a database so that tube locations are always known; however, an optional 2D DataMatrix code can be applied on each tube for redundant identification and verification. In combination with a highly controlled dry environment (0.4g water/kg air @-20°C), it has become possible to store dissolved compounds in DMSO for several years with minimal to no deterioration in sample quality. For the storage of mother solutions, REMP has added other tube formats and volumes. REMP Tube Technology™ lends itself to the assembly targeted libraries in a controlled storage environment. The REMP Sample Administration System (SAS), a modular and powerful software package, has been enhanced to cover the logistic aspects of targeted libraries. The TPM is also now offered as a compact PC-controlled device for use standalone or integrated into a third party robotic system.

The REMP Automated Plate Replicator (APR), is a system which produces large numbers of storage and screening plates of the highest quality by using the same care in minimising water absorption and exposure to air. The latest addition to this REMP product line is the nanoAPR. The nanoAPR is designed for high throughput plate replication down to the sub-µL range. Maintaining high sample integrity is supported by processing the samples under inert gas atmosphere from immediately after piercing of the mother plates until sealing of the daughter plates. Quality assurance of the non-contact pipettor is done by frequent checks through an embedded image analysis system. Carryover, DMSO consumed and the tip wash cycle times in this fixed-tip system are all minimised by a unique back-wash capability. Up to four pipettors each with 384-channel heads enable rapid processing of 384 or 1536 well plates with a daughter plate production rate of less than one minute per sealed 1536 well plate in a multi-pipetting mode.

REMP is working on an even smaller sample store to cover the needs for sample storage and retrieval of satellite laboratories or start-up companies. Like other REMP products, the Small Size Store (SSS) will be modular and scalable with a



REMP's integrated 384 tube punch (**A**) and a 96 well standalone tube punching module (**B**)

capacity of roughly 1,000 to 3,000 shallow well plates. First units will be installed in the second half of 2005.

RTS Life Science's (www.Rtslifescience.com) range of innovative automated storage solutions include SampleStore™, CompactStore™, FlexiStore™ and more recently SmaRTStore™. To help reduce the perceived bottleneck associated with tube picking, RTS has developed a high speed, high throughput, 384-tube picking system that is able to rapidly pick and place multiple single use tubes. This new technology deploys a combination of simple modelling algorithms and other innovative technologies. The result is higher throughput than that achieved by any other means. However, there is a recognition that this breakthrough may well continue to be used in parallel with the more traditional 96-tube technology, given the flexibility that this method of storage offers. RTS' 384 technology can be applied to existing RTS stores, such as SampleStore™ and our latest small storage system, SmaRTStore™, giving a competitively priced solution for both large pharma and biotech companies.

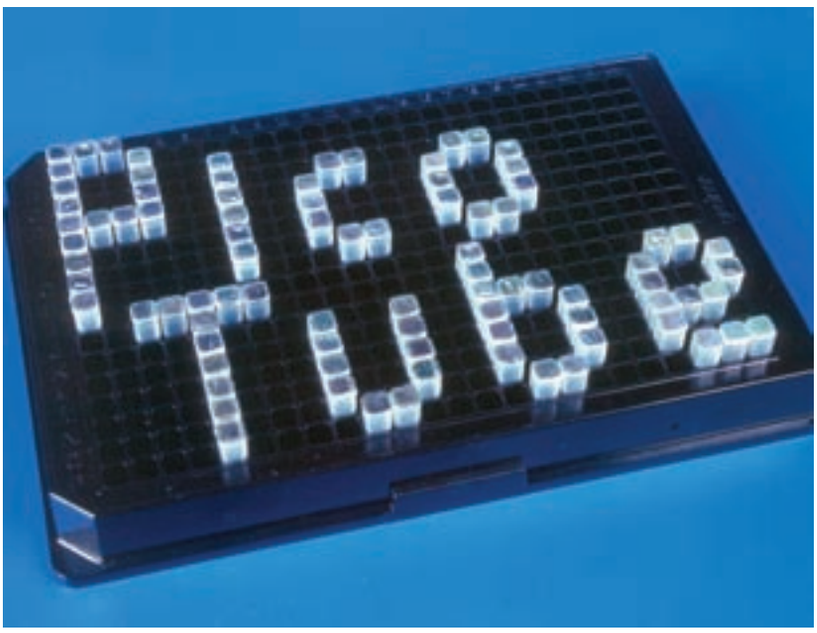
A typical RTS Life Sciences CompactStore™ which can hold up to 2,000 tubes per tray

RTS storage systems are not concerned purely with hardware. The long established d-SPRINT™ software platform has been proven as a solid basis for automated compound management solutions. This distributed software system, based around the RTS Life Science Store Inventory System (SIS), offers significant benefits in compound storage and retrieval, as well as integrated liquid handling. Thus far, d-SPRINT™ solutions have focused solely on

the automated aspect of compound management, but a recent project undertaken by RTS required the extension and enhancement of d-SPRINT™/SIS functionality into the area of manual and semi-automated solids and liquids sample processing. A number of Manual d-SPRINT™ applications have been developed, to support functions such as local bottle stores, new sample registration, solids weighing, 'un-weighable' solids processing, collation and dispatch of materials etc. These applications, intended for intensive manual usage, typically make use of touch-screen GUIs, and are founded on a configurable XML-based process route core, allowing later reconfiguration and extension.

This modular approach to compound library storage and management is available today, but there remain challenges for the future. One exciting prospect realised and developed by RTS is the closed-loop screening system (Assay Platform™), which has been developed and combined with a compound library store and a high-throughput screening system to give RTS Symphony™. Here, hits automatically generate secondary screening requests, which are then screened immediately.

The Automation Partnership (TAP) (www.automationpartnership.com) PicoTube is a 384-way tube format, suitable for 'single use' sample management. PicoTubes have a total volume of 100µL and a square section to the tube opening, which maximises access for liquid handlers. PicoTube's internal profile ensures dead volume is minimised to around 1µL. Once sample has been distributed, a seal is applied to each tube in the rack using TAP's SealPunch unit. The SealPunch also singlicates PicoTubes, ready for high speed picking in HomeBase, TAP's storage and retrieval system. PicoTubes are available uncoded or with a 2D code on the base and click into stackable SBS format racks. Increasing use of focused decks in drug discovery demands higher cherry picking rates, and HomeBase delivers this. The High Speed Picker comfortably allows 100,000 PicoTubes to be picked per 24 hour period and in duration tests at -20°C, the simple, elegant design,



Top: TAP's PicoTubes in a 384 tray, with inset of enlarged PicoTube
Above: TAP's Concerto sample management software interface

which has only a few moving parts, proves to be extremely reliable.

TAP's Concerto is flexible sample management software which condenses TAP's extensive experience in sample management into a modular, hardware independent product. TAP has managed the diverse sample data flows for more than 20 different sites over the past decade which means that Concerto comes packed with many powerful features. Oracle-based Concerto includes an ordering layer for requestors and a fulfilment layer for repository users, all accessed via the web. As requestor demands for different order types evolve, the repository team can respond by implementing new workflows using Concerto's Process Builder capability. Similarly, as novel sample handling hardware is released, Concerto's Generic Sample Storage and Generic Sample Processing interfaces can be used to integrate additional systems. Concerto avoids the need for extensive software customisation when a change is required.

Floating Tips is a development for TAP's BasePlate liquid handling module, offering variable volume sample transfer from 500nL down to 100nL in 10nL steps for 96, 384 and 1536 plates. The Floating Tip Head uses cannulae to transfer compound directly from stock DMSO solutions into assay ready plates. As the name suggests, Floating Tips avoids the issues seen with pin tools, where slight variations in plate geometry cause unacceptable CVs. Floating Tips is a simple and robust approach to a difficult application and field tests have proven the design across diverse plate types. For users with stock concentrations that require a lower transfer volume, TAP can integrate other technologies of the user's choice.

TekCel's (www.tekcel.com) TubeStore/TubeServer system is a complete automated storage and retrieval solution for 2D bar-coded microtubes and microplates. The system is designed to support end users in a screening environment with easy to use, feature-rich software and an integrated Input/Output device, the TubeServer. The TubeStore stores and organises 2D bar-coded tubes in their original racks at user-selected temperatures and atmospheres. The system presently supports 1.4mL, 0.7mL, 0.5mL and 384 microtubes from major vendors. Cherry picking of the tubes takes place within the storage environment. The TubeStore's unique design allows all robotic movements to be concentrated in the front of the unit and multiple tasks are handled simultaneously. The TubeStore's high-speed picking unit is design to reduce the travel distance of any single tube from source rack to destination rack. Raw picking speeds of more than 1,000 tubes per hour have been reached. One TubeServer can support up to five TubeStore units in a lab.

The TubeServer provides fast, easy manual and robotic access to the storage environment. There are 40 positions for tube racks or microplates on the manual door system. The door positions also double as a thaw rack if the samples are kept frozen. The integrated hand-off arm allows for easy integration to third party liquid handling

devices. The TubeManager software comes standard with features such as defragmentation, self audit and exclusion mapping. As the use of localised storage systems moves further away from the centralised compound management group, easy to use software appears to be playing a more critical role in the adoption and acceptance of such systems. The system will defrag on demand and report to the user's LIMS system the new tube locations. When running a self audit, every rack or plate will be brought to the TubeServer, the 1D (rack) and 2D (tube) bar codes are confirmed and then the samples are returned to storage. You can also create an exclusion map to create empty positions within a tube rack. Simply associate the map with the pick list and the system will apply the exclusion criteria to all destination racks in that request.

TekCel is continuing to work with users to take full advantage of the hardware capabilities and software features for the TubeStore platform. One TubeStore system can support the most demanding cherry picking operations delivering more than 120 completed racks of pre-picked 96 well tubes an hour.

TTP LabTech (www.ttplabtech.com) comPOUND® is an extremely reliable high-density sample store with a track record proven at customer sites over the past three years. Robots and conveyor belts can be bulky and unreliable at sub-zero storage temperatures, so comPOUND's -20°C freezer is designed with a pneumatic delivery system. It takes only five seconds to cherry-pick any microtube out of 100,000 and deliver it to the front of the store. It can also whisk microtubes from a storage area to a lab, even if the two are on different floors. comPOUND's reliability is enhanced because it has very few moving parts, and the majority of those are located outside the temperature-controlled chamber for easy maintenance access. There are no robot grippers to cause failure, and microtubes are contained in the module's inert atmosphere right up to the point of delivery.

comPOUND® is capable of handling rapidly changing project needs because the modular design means a growing company does not have to invest in a large, cumbersome system early on. Multiple modules can be connected, and relocated, easily to allow the library to grow as the company expands. comPOUND® can also be interfaced to comPILER®, an automated store-to-plate processing system capable of handling more than 15,000 microtubes in an eight-hour day without operator intervention. comPILER® can connect up to 12 comPOUND® stores, allowing rapid

access to a library which can range from 100,000 to 2.4 million samples. comPILER® and comPOUND® make a self-contained system that does not require special laboratory conditions or bespoke storage facilities.

Current opinion is that sample integrity is compromised mainly during freeze/thaw cycles. This is due to uncontrolled water uptake into DMSO stocks during cooling causing precipitation. If you can effectively control DMSO hydration, it may be difficult to experimentally show an adverse effect of freeze thaw cycles[3]. TTP Labtech and Wyeth recently ran a series of tests on comPOUND® and comPILER® to validate this assumption[4]. To test the effectiveness of these systems to ensure sample integrity, Wyeth took samples from frozen storage in comPOUND® through the comPILER® processing cycle and back to storage 10 times. Before storage on each cycle Wyeth measured the percentage of water

TekCel's TubeStore and TubeServer system

uptake in DMSO by Karl Fisher titration. The results (**Figure 11**) demonstrate that water uptake is negligible when samples are processed by comPILER® highlighting the effectiveness of the comPILER®'s argon 'plug' overlay to prevent water ingress after repeated sample processing.

As a next step, TTP LabTech is developing a method to remove the cumbersome process of plate reformatting, and allow users to take sub-µL volumes from the retrieved storage microtube direct to an assay-ready plate, without intermediate dilution. TTP has an advantage in this area in that its unique mosquito® low volume liquid handling disposable micropipettes can pierce tube caps, aspirate from the tube and then dispense into 96, 384 or 1536 plate formats. This should facilitate assay miniaturisation, conserve samples and reduce the time taken to prepare assay-ready plates, while fully maintaining sample integrity.

TTP Labtech's comPILER® integrated with three comPOUND® storage units

The MatriStore™ by **MatriCal** (www.matrical.com) is an automated compound management system designed with speed, capacity and most of all, flexibility in mind. Using 'off the shelf' components it gives the customer not only an extremely reliable product, but one that is very cost effective. The system is climate controlled from room temperature to -60°C and is ideal for storage of chemical compounds, bacteria and other micro-organisms, DNA, plasmids, cosmids and more. One system can support multiple sample formats such as MatriCal's proprietary 96 and 384 2D MatriTube™ storage mini-tubes, 96 to 1536 microplates, 9 to 25mm dram vials, etc. The MatriStore™ has a small foot print of 3m x 5m and is available in a range in heights from 3m to nearly 10m in height. The 3m system has a total plate storage capacity of approximately 30,000 plates; 10m system stores 130,000 plates. A smaller 10,000 plate system called the Matri-MiniStore has recently been designed. Speed is achieved both by the use of high density storage trays (each with a capacity of 520 384-well plates) as well as by the use of a SCARA robot. Statistically, there will be two picks per 384-well plate or more than 1,000 picks per tray. It takes 17 seconds to pull a tray and 17 seconds to put it away and a further 1.5 seconds per tube for the SCARA robot to pick a sample. Picking rates of 20,000 picks per 20 hours have been accomplished. Flexibility is achieved by the use of plastic tray inserts in which different sample types can each be stored in separate tray inserts, all within one system. Picking tools on the robots change on the fly, achieving maximum flexibility

and allowing different samples types to be retrieved sequentially from their respective tray.

The MatriServer™ graphical user interface (GUI) written in Visual Basic.NET provides an intuitive platform to submit orders and verify the status of pending requests. Users can be trained in the basics of entering or retrieving samples from the MatriStore™ in less than 20 minutes. The GUI also offers paging and e-mail service to alert users of completed orders. The MatriServer™ has a reporting component, in which XML reports are generated upon completion of an order. This reporting component can easily be integrated with your existing compound management software. MatriCal is currently expanding its offering to the handling of solids including powders, tars and oils, and the integration of liquid handling.

The development of **Discovery Partners International**'s (DPI) (www.discoverypartners.com) Universal Store drew on core sample management competency from its Discovery Chemistry Division and through project experience such as development of the small-molecule repository for the National Institutes of Health's Molecular Libraries Initiative. The Universal Store addresses the chief concerns of modern compound management in an affordable, large capacity, small footprint next generation modular system that can easily be configured to meet virtually any drug discovery sample storage need. The Universal Store is intrinsically responsive to the growing variability in sample containers by accommodating simultaneous storage of many container types such as tubes, vials and microplates. The small footprint (3.7m x 4m) conserves precious floor space and storage capacity is configurable to accommodate from 100,000 to 17,000,000 samples by increase in height (2.4m to 7.3m) and selection of container type. Sample integrity is maintained by keeping all storage and cherry picking sample manipulation under frozen and inert atmospheric conditions. Individual sample tracking, intelligent storage and handling management, and exceptionally high cherry picking rates alleviate bottlenecks in cherry picking and sample retrieval currently experienced by CM groups. Additionally, multiple freeze-thaw cycles, a problem implicated in compound solubility, are significantly reduced as only the samples of interest are picked, defrosted and delivered. Configuration versatility makes the Universal Store an ideal platform for achieving a myriad of storage objectives and formats including dividing sample storage among multiple sites; long term and working storage; dedicated HTS and MedChem site storage; and duplicate back-up storage for disaster recovery. DPI also utilises the Universal Store in its Discovery Chemistry group to offer innovative service models of off-site or on-site sample storage, retrieval, distribution, and quality control.

Conclusions

As CM groups struggle to maintain the integrity of their compounds over the coming years, flexibility is essential in order to enable new sample processing routes to be integrated into the list of

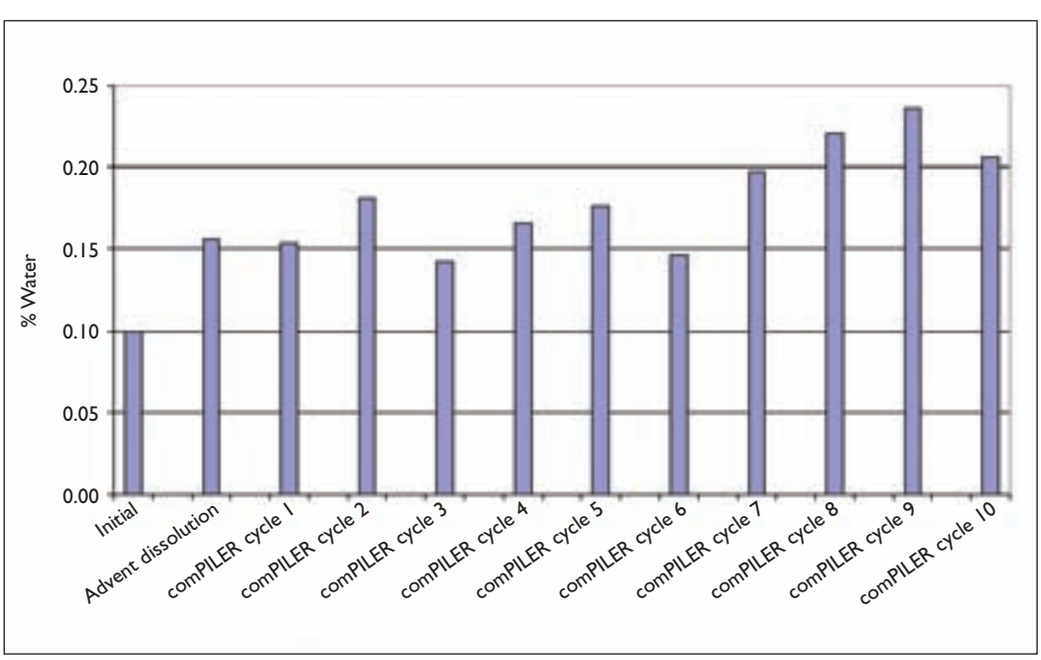


Figure 11
Shows the water content of DMSO as supplied (0.1%) and the subsequent uptake during dissolution of solid material and 10 processing cycles in and out of storage using comPOUND/comPILER (TTP Labtech), the final water content did not exceed 0.15%

compatible processes. Integration of new technologies such as non-invasive (acoustic) sample transfer, volume/stock measurement, sample monitoring and life cycle planning are all subjects that will require further attention. The next big challenge will be to provide a dynamic, in most cases increasing, sample collection to more full diversity and targeted screens, with faster turnaround times (increased picking rates), in higher plate densities and in smaller (sub-microlitre) sample volumes. DDW

Dr John Comley *is Managing Director of HTStec Limited, an independent market research consultancy whose focus is on assisting clients delivering novel enabling platform technologies (liquid handling, laboratory automation, detection instrumentation and assay reagent technologies) to drug discovery. Further information on accessing the market report 'Compound Management Trends 2004' can be obtained by visiting www.htstec.com or e-mail surveys@htstec.com to receive a free copy of the Report's Executive Summary and Table of Contents.*

References

1 Carmody, C et al (2004). The use of chemiluminescent nitrogen detection (CLND) to investigate the actual concentration of liquid samples in corporate compound collections. Poster presented at IQPC Compound Management and Screening Meeting, London, 25-27 May 2004.
2 Lane, S et al (2004). Towards Single Calibrant Quantification in HPLC – A Comparison of Three Detection Strategies: Evaporative Light Scattering, Chemiluminescent Nitrogen, and Proton NMR. Poster presented at ISC 04 Meeting, Paris, 4-8 October 2004.
3 Lipinski, C (2005). Compound precipitation from DMSO and the synergy between water uptake and freeze/thaw cycles. Oral Presentation at LRIG Meeting, January 2005.
4 Longden, M, Morin, J and Mobilio D (2004). Integration of Sample Management & HTS at Wyeth. Oral Presentation at LRIG Meeting, Somerset, NJ, 14 January 2004.

Discovery Partners International Universal Store