Best Practices in Compound Management for Preserving Compound Integrity and Accurately Providing Samples for Assays

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Preserving the integrity of the compound collection and providing high-quality materials for drug discovery in an efficient and cost-effective manner are 2 major challenges faced by compound management (CM) at Bristol-Myers Squibb (BMS). The demands on CM include delivering hundreds of thousands of compounds a year to a variety of operations. These operations range from single-compound requests to hit identification support and just-in-time assay plate provision for lead optimization. Support needs for these processes consist of the ability to rapidly provide compounds as solids or solutions in a variety of formats, establishing proper long- and short-term storage conditions and creating appropriate methods for handling concentrated, potent compounds for delivery to sensitive biological assays. A series of experiments evaluating the effects of processing compounds with volatile solvents, storage conditions that can induce freeze/thaw cycles, and the delivery of compounds were performed. This article presents the results of these experiments and how they affect compound integrity and the accuracy of compound management processes. (*Journal of Biomolecular Screening* 2009:476-484)

Key words: compound management, drug discovery, storage conditions, sample distribution, volatile solvent transfer, hit identification, lead optimization

INTRODUCTION

HIT IDENTIFICATION BY PRIMARY SCREENING of compound collections forms the basis of compounds developed through the lead optimization process.[1] This large and diverse set of compound entities that comprise the primary screening set can be stored for a number of years and subjected to a variety of compound-handling processes. Therefore, construction and preservation of compound sets using validated processes are of paramount importance in generating compounds that could potentially make their way into the development pipeline.[2]

This article highlights in-house experiments that were performed to investigate the validity of compound management processes in terms of compound integrity and accuracy. The experiments focus on (a) process flows that include the transfer of compounds, (b) storage within a variety of conditions, and (c) compound delivery for biological screening.

Transfer of large (hundreds of thousands) numbers of solid samples continues to be a challenge for compound management (CM). Weighing free-flowing powders can be automated to some extent, but the provision of non-powder-like samples is a time-consuming and manual process. To alleviate this problem, CM adopted the volatile solvent transfer (VST) process to transfer compounds into vials or plates. This process was historically used in the combinatorial chemistry arena for a number of years. The VST process, which is especially useful when a dry compound is a film, gum, or oil, involves dissolving the compound in a solvent, transferring an aliquot to the desired output container, and then evaporating the solvent. In addition, this process has the advantage of being able to use automated liquid handlers for processing large sets of samples. As a result, VST processes are now used for a variety of compound management processes, including the creation of large premade compound sets.

Like other companies in the industry,[3,4] Bristol-Myers Squibb (BMS) uses premade compound plates for use within hit identification screens. The storage period of these DMSO solutions typically ranged from 3 to 5 years. The determination of whether contaminants were leaching from the plastics

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into the solutions during this timeframe was important. With advancements in miniaturization technologies for liquid handling and screening,[5,6] the amount of sample required decreased, and the storage period was extended. To preserve compound integrity and still maintain a format that is amenable for quick compound supply, the process needed further evaluation.

In addition to the long-term storage investigations, short-term (<1 year) storage conditions and its potential effect on compound integrity required examination. This became necessary because CM support was extended to include delivery of assay-ready plates for lead optimization assays. Unlike the compound archive where compounds are stored as 3-mM solutions at 4 °C, this process required the storage of solutions up to 20 mM. A review of the literature[7-9] for storing compounds at high concentration indicated storage at room temperature as an option. This storage practice was investigated by using structural integrity analysis during the lead optimization process.

A fundamental component associated with the lead optimization process is performing the assay at accurate and well-defined concentrations. When compounds are solublized in DMSO and reformatted into source plates within 24 h to assay initiation, there is a need to preserve the solution's concentration during this short timeframe. An option to minimize water absorption by DMSO is to thermally seal plates. Water absorption auditing technologies[10] have enabled the review of the efficacy of thermally sealed plates in preventing water uptake.

Even with preserving the source plate's concentration, the challenge of handling highly concentrated, potent compounds during assay-ready plate creation remains. The accuracy of submicroliter volume transfers is easily validated by liquid chromatography/mass spectrometry (LC/MS), nuclear magnetic resonance (NMR), and fluorescent/absorptive dyes. However, the analytical and dye techniques are not sensitive enough to indicate whether a tip-washing method is sufficient to negate a biological response to a potent compound. To determine whether a tip wash method is sufficient, one must use the biological assay to establish and reevaluate the validity of the method.

Confirming the validity of compound management processes—specifically compound handling, storage conditions, and compound delivery—contributes toward establishing an increased confidence in the data obtained from the lead optimization and hit identification processes. This is due to having a detailed understanding of how each process affects the integrity of a compound and the accuracy of the delivered product.

MATERIALS AND METHODS

Compound handling

VST solvent removal during the evaporation step and accuracy of compound transfers analysis. For this study, a proprietary sample was chosen based on the following criteria: known purity, soluble in 1:1 dichloroethane/methanol (DCE/MeOH) and DMSO, with an NMR peak that did not overlay with solvent peaks. Approximately 5 mg of material was manually weighed into fifteen 1-dram vials. Using a TECAN Genesis instrument (TECAN, Männedorf, Switzerland), DCE/MeOH and DMSO were each transferred into 3 vials to dissolve the compound. Subsequently, 3 μmol of material was transferred into a 96-well block polypropylene deep-well block. Using a calibrated hand pipette, DCE/MeOH and DMSO were added to a separate set of vials with a 3-μmol transfer into the block. The deep-well block was evaporated (ThermoSavant Explorer, Thermo Scientific, Waltham, MA; 2-h runtime, 1-h heat/lamp, 45 °C chamber temperature, max vacuum, full ramp). After evaporation, the samples were resuspended using 220 μmol of 75% d-DMSO/25% CDCl3 with a 1:1 proton ratio of 3-(Trimethylsilyl)propionic-2,2,3,3-d4 acid (TSP). Using a calibrated hand pipette, the remaining 3 vials were dissolved using the mixture above. After dissolution, 3 μmol of material was manually transferred into the deep-well block.

Using flow-NMR, each sample was subjected to 64 scans on a Varian 600-MHz spectrometer (Varian, Palo Alto, CA). Data were processed using ACD software and an autoprocessing macro. Quantification was based on the integral of an isolated signal at 5.30 or 4.40 ppm against TSP (0 ppm).

Evaporation chamber temperature effect on compound integrity. Fifty-six chemically diverse compounds that displayed decreasing potencies across multiple assay instances were selected for this investigation. Using a constant sample injection amount (12.5 μg/5 μL DMSO), the samples were monitored for compound loss or degradation by high-performance liquid chromatography–ultraviolet (HPLC-UV) or HPLC–mass spectrometry (HPLC-MS) analysis. The area under the peak for the samples was measured in milli-absorbance units (mAUs). This involved starting with a fresh (T_0) sample, followed by processing it through 9 sequential centrifugal evaporation cycles (i.e., T_1-T_9) at 45 °C and 55 °C temperature settings. HPLC peak areas for the major peak in each sample (area in mAUs, area percent of total) were recorded. For compounds that showed little or no UV absorption at 254 nm, UV detection at 220 nm or MS detection was used. Sample sets were run in duplicate, bracketed with blank runs and caffeine external standards for HPLC system verification. Upon completion of the HPLC analyses for all compounds, the UV peak area values for the major peaks were compared to those obtained for the (T_0) sample to determine the percent loss of sample.

Storage conditions

Leach test analysis of storage labware for long-term storage. Plates (REMP, polypropylene, TECAN 23490-104) were filled with 100 μL of deuterated DMSO. The plates were sealed

(06643.001, Velocity11, Santa Clara, CA) and stored at 4 °C. Over the course of 3 years, plates were periodically removed from storage, thawed, and analyzed via proton NMR analysis with a Bruker DRX 500-MHz spectrometer (Bruker, Billerica, MA).

Long-term storage of dry compounds within source plates. To produce meaningful comparison data, we selected 96 and 229 compound sets with a range of activities and chemotypes from hit identification screens for use in the experiments. The compounds were dissolved to 3 mM in 99.9% grade DMSO (Mallinckrodt Backer, Inc., Phillipsburg, NJ), and a constant volume (20 µL) of the solution was transferred to each well of a 384-well plate (REMP) in duplicate. One copy of the plate was kept in liquid form to serve as a control. The second copy was dried down using an evaporator (ThermoSavant Explorer; 2-h runtime, 1-h heat/lamp, 60 °C chamber temperature, max vacuum, full ramp). The control copy was subsequently diluted with 10 µL of DMSO to create a 2-mM source plate. The dried-down copy was dissolved to 2 mM using 30 µL DMSO and shaken on an orbital platform through ten 30-s cycles of varying orbital directions. Compounds from both sets of plates were assayed in triplicate for activity in biological assays.

A fluorescence-based protease assay was run using a commercially available substrate, (Cy3)(S)EVNLDAEFK(Cy5Q), from Amersham Biosciences (Fairfield, CT). The cy3/cy5-modified 9 amino acid was cleaved by recombinant beta-site amyloid precursor protein cleaving enzyme (BACE) that was developed in house in Chinese hamster ovary (CHO) cells. The final assay conditions consisted of sodium acetate (pH 4.5, 50 mM), sodium chloride (230 nM), BACE (30 nM [20 ng]), (Cy3)(S)EVNLDAEFK(Cy5Q) (1 µM), and DMSO (3.5%). The plates (Corning #3575) were incubated for 1 h at 25 °C and then read on an LJL Analyst (535 nm EX and 595 nm EM).

Cyclic AMP (cAMP) accumulation assays were performed using the human retinoblastoma cell line, Y79, which endogenously expresses type I corticotrophin-releasing factor (CRF) receptors (CRF-1R). Briefly, cells were removed from tissue culture flasks and resuspended at 1×10^6 cells/mL in calcium- and magnesium-free Hank's buffered saline solution (HBSS; Invitrogen, Carlsbad, CA) supplemented with 25 mM HEPES and 1 mM 3-isobutylmethylxanthine (IBMX). REMP plates containing 20 µL of cell suspension were preincubated with 1 µL of test compounds. cAMP accumulation was initiated by the addition of 20 µL of HBSS containing 25 mM HEPES, 1 mM IBMX, 0.005% Triton X-100, 1% (w/v) bovine serum albumin (BSA), and 5 nM CRF peptide. After a 30-min incubation at room temperature, cAMP accumulation was terminated by the addition of 20 µL of 1.5% Triton X-100. Low-volume, 384-well plates (ProxiPlates-384F, 6006260, PerkinElmer, Waltham, MA) were created containing 4 µL of the cellular lysate. Cellular cAMP was quantified using a homogeneous time-resolved fluorescence (HTRF) assay kit (CISbio US, Bedford, MA). Stock solutions of the HTRF cAMP tracer, cAMP-XL665, and europium cryptate-labeled anti-cAMP antibody were prepared according to the manufacturers' instructions. Tracer and antibody were diluted 1:20 in HBSS containing 25 mM HEPES and 0.8 M potassium fluoride, and 2 µL each of the diluted reagents was added to cellular lysate. Following a 1-h incubation at room temperature, assay plates were read on a Packard Discovery HTRF plate reader. Inhibition of CRF-induced cAMP accumulation by the test compounds was normalized to the amount of inhibition produced by a known small-molecule CRF-1R antagonist.

Freeze/thaw analysis of compounds stored for <1 year by LC/MS. Compounds at 20 mM in 100% DMSO were stored in screw-capped, 1-dram vessels (60910D1, Kimble Chase, Vineland, NJ) at room temperature, ambient humidity, and dark conditions for an initial 2-week window. The amount of DMSO used for dissolution ranged from 50 to 3500 µL, but the average vessel contained approximately 280 µL. The compounds were subsequently stored in the dark at 4 °C for up to 6 months. Any requests for work after the 2-week timeframe involved retrieving the samples and allowing them to thaw to room temperature. If the sample passed a visual inspection for solubility, then the processing request was fulfilled.

Compounds were analyzed for structural integrity in 96-well plates (PCR-96-FS-C, Axygen, Union City, CA) as 3-mM DMSO solutions. The 3-mM compound solutions were diluted 1:14 with a cocktail solution containing equal volumes of acetonitrile, isopropanol, and Milli-Q H_2O. The final compound concentration was 214 µM. Samples were analyzed at this concentration by LC/MS using a Waters XBridge C18, 5 µm, 2 × 50 mm, with a Waters Sentry 2.1-mm guard column at a flow rate of 1 mL/min, using a 5-µL sample volume injection on a Finnigan Ion Trap mass spectrometer (Finnigan, San Jose, CA). Purity was determined by quantitative analysis of the area under the curve (UV @ 220 nm) for the specific compound molecular weight.

Water uptake in lidded and sealed plate analysis. Plates (REMP, polypropylene, TECAN 23490-104) were filled with 40 µL of 211 µM New Fuchsin dye (N8652, Sigma, St. Louis, MO) in DMSO. Plates were either lidded or thermally sealed (06643.001, Velocity11) and incubated at 26 °C, saturated (1 atmosphere) humidity. After 16.5 h, 20 µL was transferred into plates (P-05525, Labcyte, Sunnyvale, CA) and audited on Labcyte's Echo 550 for percent DMSO per well. Absorbance was then measured at 490 nm using PerkinElmer's HTS7000.

Compound delivery

Using assays to validate compound processing: carryover determination. In this experiment, a series of concentration-response curves were generated for potent compounds. The highest concentration of compound was located in columns 1

and 11 of a REMP plate. Two additional REMP plates were made with just DMSO solvent in the wells. A VPrep (02318.102, Velocity11) liquid handler equipped with an auto-filling DMSO reservoir station and a 384-chimney microwash station was used for all transfers from the REMP plates into the assay plate. Prior to compound handling, a DMSO plug and air gap was preaspirated into the tip. After the compound dispense, the DMSO plug was purged into the waste section of the microwash station. Full tip volume aspirations of water from the microwash chimneys were aspirated and subsequently dispensed into the waste section. The cycle of aspirations and dispensation was repeated 7 times. Following the water wash, a full tip volume aspiration of DMSO was made from the DMSO reservoir followed with a dispense into the waste section of the microwash station. This cycle was repeated 3 times. The entire process was repeated for each source plate transfer. The order of processing the REMP plates was as follows: concentration response curve, DMSO plate, and DMSO plate.

RESULTS AND DISCUSSION

Compound handling

The incorporation of VST processes for the transfer of solid samples introduced efficiencies in throughput. Typically, a full-time employee can manually weigh 300 to 400 samples a day, depending on the physical properties of the compound. Use of the VST process can increase this throughput to about 1000 samples per day per liquid handler. In addition, the VST process has the ability to easily handle compounds that are not free-flowing powders and for the creation of plates that contain dry compound films. However, the VST process should not be used when compounds must maintain a crystalline nature because this process results in the generation of compounds that possess the physical characteristics of amorphous films or oils. Although VST forms the basis of compound purification and transfer in combinatorial chemistry,[11] some concerns remained in its use in compound management processes. Total solvent removal, the use of heat during the evaporation step coupled with its effects on compound integrity, and the accuracy of material transfer using volatile solvents by automation needed review.

Ineffective removal of solvent during VST processing results in erroneous weights being attributed to the compound vessel. This error is magnified with successive iterations of compound transfer from the same vessel. More important, the resulting concentration of the compound in biological assays will be inaccurate, suggesting a higher concentration than actually present.

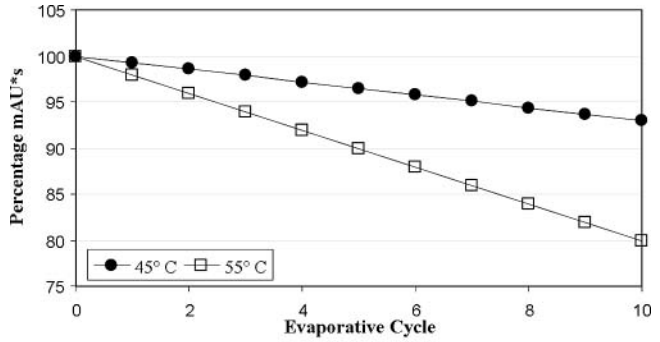
To evaluate the effectiveness of solvent removal during the evaporation process, we undertook NMR analysis after compounds were dissolved in either DCE/MeOH or DMSO, transferred, and dried down. In some cases, during the initial experiments, total solvent removal was difficult to ascertain because of the overlap of solvent peaks with the compound peaks. To alleviate this issue, a single compound of known purity and no overlay with the test solvent peaks was chosen for further detailed evaluation. Subsequent analysis by NMR revealed that no solvent remained based on the limits of detection. Although this study indicated that the existing evaporation methods were sufficient to remove volatile solvents, individual compound properties could affect the method's success. As a safeguard, BMS includes a check-weigh step after the evaporation cycle. This step compares the postevaporation weight of the sample to the expected weight to indicate whether there may be trapped solvent. If a mass discrepancy is observed, then the evaporation step is repeated.

Another condition influencing the accuracy of compound transferred by VST is whether the automation can handle the volatile solvents. An extension of the above experiment included using the NMR to quantitate the amount of material transferred within the VST process. When compared to a calibrated hand pipette, the VST process for a highly volatile solvent of DCE/MeOH showed similar transferring capabilities. The 2 processes yielded 118% ± 15% and 109% ± 10% of the expected amount, respectively. Transfers for a less volatile solvent, DMSO, exhibited comparable results. The transferred mass via VST was 114% ± 7%, whereas the hand pipette was 110% ± 14%.

In addition to the accuracy of the VST process experiment, the temperature of the evaporation chamber was assessed for its potential affect on compound integrity. Within this experiment, a chemotype diverse set of 56 compounds that displayed decreasing potencies across multiple assay instances was selected. The compounds were processed through 10 evaporation cycles within 2 test conditions: 45 °C and 55 °C chamber temperatures.

As seen in **Figure 1**, a 1-sided paired *t*-test ($t = 2.10$, 1-sided p-value = 0.0200, $df = 55$) on the averaged estimated slopes from regression analysis of HPLC-UV data was used to determine a 0.7% compound loss per cycle at 45 °C. At a higher temperature, 55 °C, compound sample loss per cycle was determined as 2% per cycle. It should be noted that the exact mechanism of compound loss (i.e., chemical vs. physical) was not determined in this study. However, on average, there was a 1.33% difference in the reported compound integrity between the 2 chamber temperatures. Thus, if a compound undergoes 10 evaporation cycles, the compound loss at 55 °C will be 13.3% greater than the compound loss at 45 °C.

As seen in **Table 1**, of the 56 chemotypes tested, most chemotypes (44-49) did not exhibit significant degradation or loss (<3%) per VST cycle even at the higher temperature. Clearly, a set of other chemotypes (7-12) was more susceptible to temperature (>3%). Even though compounds could be evaporated without applying heat, the time required for evaporation would be dramatically increased, especially for DMSO solutions. In light of balancing the time for evaporation and the loss



Slopes (% per cycle)	Mean	Std. Error
45°C	-0.7050	0.3653
55°C	-2.0339	0.7864
Mean Difference	1.3289	0.6316

FIG. 1. Average compound loss observed for 56 compounds subjected to multiple evaporation cycles at 45 °C or 55 °C. Results were determined via high-performance liquid chromatography coupled to ultraviolet or mass spectrometry detection.

Table 1. Distribution of 56 Chemotypes with Greater Than or Less Than a 3% Degradation Loss per Evaporation Cycle at 45 °C or 55 °C Chamber Temperatures

Temperature	*Total Number of Samples*	<3% Degradation per Cycle	>3% Degradation per Cycle
45 °C	56	49	7
55 °C	56	44	12

of sample at higher temperatures, the use of 45 °C for evaporation was implemented.

Storage conditions

Maintaining compound integrity of stored compounds includes a variety of storage containers (plates and vials), conditions (4 °C and room temperature), and time periods (greater or less than a year). The breadth of conditions is required to satisfy the diverse support needs of hit identification and lead optimization efforts.

Hit identification support needs include the storage of compounds in premade compound sets. Plates are premade to rapidly provide the compounds to a screen when requested. Historically, these compound sets were kept as DMSO solutions within 4 °C storage conditions. Because of the length of storage, BMS was concerned about the potential of plastic or manufacturing materials leaching into the solution. These contaminates could inadvertently influence the biological mechanism[12] and lead to ambiguity in the data. To evaluate whether polypropylene REMP plates could be used for storing compounds within these conditions, BMS periodically analyzed DMSO samples from plates by proton NMR. The result of this multiyear study

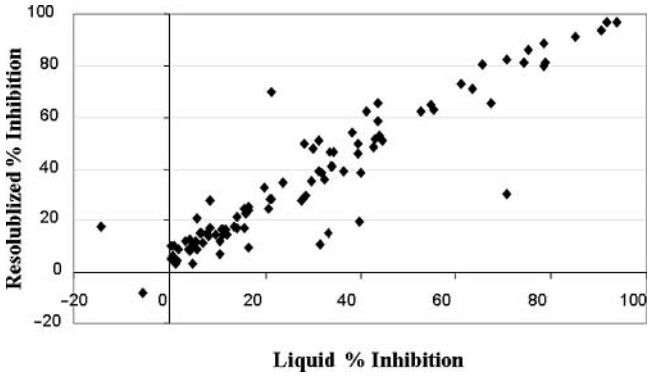


FIG. 2. Percent inhibition comparison of liquid and resolublized methods for a 96-compound set.

demonstrated that the only visible peaks in the spectrum were that of DMSO and water. This indicated that for the anticipated conditions and storage lifetimes of the DMSO solutions, no foreign materials were detectable by NMR analysis.

However, as the shelf-life of the premade compound collection sets was extended due to miniaturization, BMS assessed whether evaporation of the DMSO solvent coupled with resolubilization of compounds in plates would negatively affect biological data. If this process was valid, then compounds could be stored in a less reactive, dry state and thus maintain compound integrity.[13] A set of 96 chemically diverse compounds with a range of inhibition profiles (0%-100%) was selected from a primary screen to compare the historic process, where compounds remained in solution, to the proposed evaporation and resolublization process flow. In order to not skew the data to look only at active compounds, a range of chemotypes with differential activity were chosen since this is more reflective of the hit identification process. The comparison of inhibition profiles from this experiment is shown in **Figure 2**. In this assay, a compound was considered active when the percent inhibition was greater than 30. Of the 42 compounds that met this criterion in the historic process, 10 compounds (7%) appeared to have lost activity in the resolubilized process. On the other hand, the overall average activity of the remaining resolubilized compounds was slightly higher (8%). Statistical analysis of the data yielded a concordance correlation coefficient[14] of 0.931, which indicates a high agreement between the two test methods. These results signify that the resolublization process does not have an adverse effect on the compounds because they generated similar biological results as the samples from the control process. To confirm these findings, we repeated the experiment in a different assay with a set of 229 compounds with a range of inhibition profiles. In this assay, a compound was considered active when the percent inhibition was greater than 54. In this instance, of the 131 compounds that met this criterion in the historic process, 10 compounds (7.8%) appeared

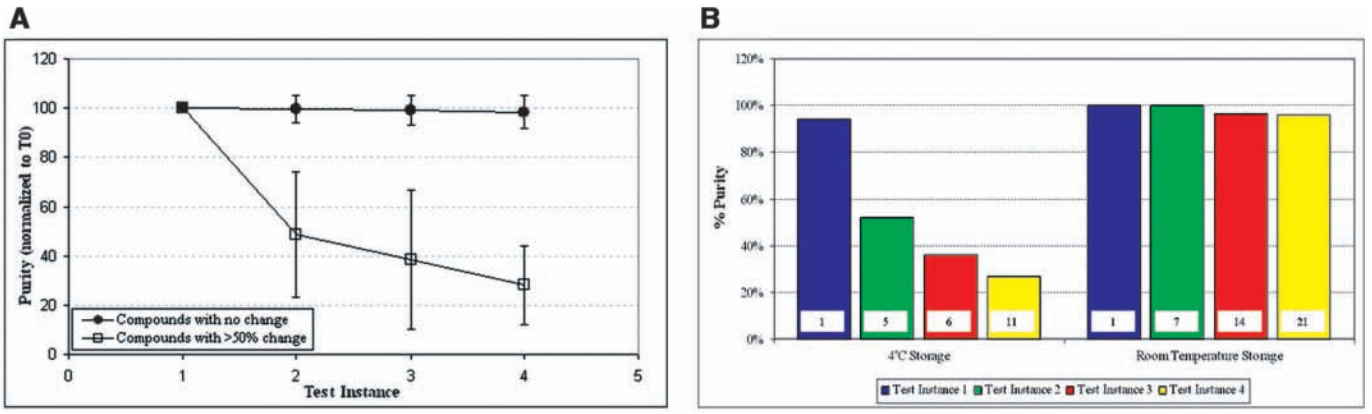


FIG. 3. (**A**) Average purity of compounds with no change (352 compounds) and >50% change (10 compounds) across multiple testing instances as detected by liquid chromatography/mass spectrometry. (**B**) A comparison of the observed loss in purity for the same chemotype when stored at 4 °C (freeze/thaw) versus room temperature. The length of storage is represented by the value at the bottom of each test instance bar.

to have lost activity in the resolubilized process. For this experiment, the concordance correlation coefficient was determined to be 0.952. On the basis of these results and to preserve compound integrity, the process of drying and reconstituting source plates was implemented for the long-term storage of pre-made compound sets.

In contrast to long-term storage of samples for hit identification efforts, the lead optimization process requires short-term (<6 months) storage of compounds. Processing of compounds through structure-activity relationship (SAR) and structure-liability relationship (SLR) assays requires the rapid generation of data to progress samples through the drug development cycle. In this process, compounds are solubilized up to 20 mM in DMSO and tested in multiple assays to generate a dossier of information. The initial storage process involved keeping the vials at room temperature for 2 weeks. This was done to avoid freeze/thaw cycles while the compounds were undergoing the initial battery of SAR and SLR testing. After the 2-week period, the compounds were transferred to 4 °C for storage. Compounds were retrieved from storage and thawed only if additional testing was required.

As part of the lead optimization process, structural integrity analysis was included each time a compound was tested for a biological assay. The gathered LC/MS data showed instances where freeze/thaw cycles affected the purity of samples. As shown in the **Figure 3A**, certain chemotypes are more susceptible (>50% decrease in purity) to freeze/thaw cycles, whereas others are not. The decrease in purity affected 3% of the 362 samples analyzed. It should be noted that this represented only 1 of the 11 chemotypes analyzed for a single program. Loss of sample could have been a result of degradation or precipitation caused by water uptake while processing the frozen sample, although no water content measurements were obtained for these samples. For the other 4 programs reviewed in this study, at least 22 different chemotypes were tested. None of them exhibited a decrease of purity after the freeze/thaw cycle. Because it is not possible to predict the degradation behavior of chemotypes toward freeze/thaw cycles, room temperature storage was extended to 6 months while the compounds existed in 20-mM solutions. After instituting the change in storage conditions, structural integrity data were reviewed again. No decrease in purity was seen for samples of the *same* chemotype when stored at room temperature. A comparison of the observed loss in purity for the same chemotype when stored at room temperature versus 4 °C (freeze/thaw) is shown in **Figure 3B**. In fact, no compound degradation was observed for all other lead optimization programs containing a variety of additional chemotypes.

Although CM creates plates for hit identification and lead optimization that are used within 24 h, there is a need to preserve the integrity of the solution during this time period. Large-volume plates (generally 4-30 µL) are used for the creation of source plates for controlled response curves. Assay-ready plates are generated just in time and typically contain small volumes (<1 µL). In both instances, absorption of water by DMSO is a concern. Concentration can be altered due to the volume changes or induced precipitation of compound. To mitigate water uptake, we evaluated thermal sealing of plates. DMSO plates were audited for percent water 16.5 h after creation. The timeframe is representative of the elapsed time between plate creation and testing in an assay. The results of this experiment are shown in **Figure 4**. The figure depicts the change in percentage of water in each well of the plate for a sealed and unsealed plate. Color coding, based on a numerical value obtained by acoustic auditing, is used to help visualization.

As can be seen in **Figure 4**, the thermally sealed plate had minimal water uptake (0% ± 0.2%), as shown by uniformity in color across the plate. However, in contrast, after only 16.5 h, the unsealed plates (**Fig. 4**, lidded plate) show a heat map with a large amount of variation. In this case, water uptake averaged 4.1% ± 3.9% across the entire plate. In addition, there was also

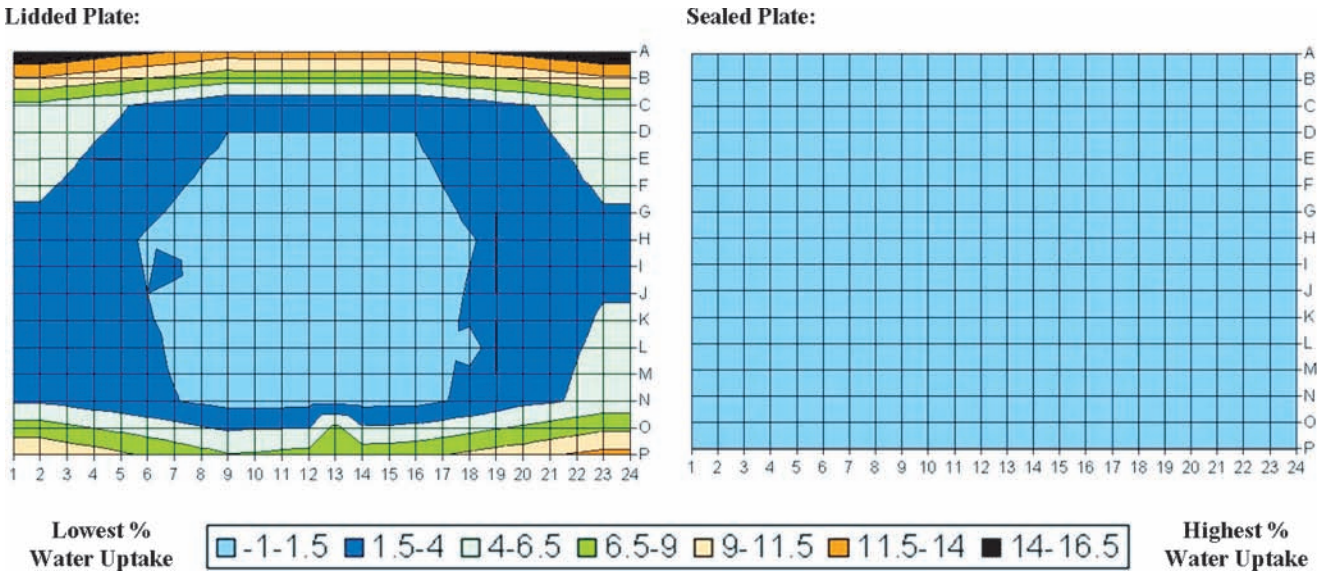


FIG. 4. Well heterogeneity seen during water uptake of lidded and thermally sealed plates that were incubated in a 26 °C, saturated humidity (1 atmosphere) environment. Results were obtained using acoustic auditing.

a 3.2-fold difference of percent water between the perimeter and interior wells with a greater percentage along the periphery. Clearly, water uptake along the plate edges is much greater than in the interior of the plate. Therefore, incorporating thermal sealing of plates enhanced the preservation of solution integrity.

Compound delivery

In the creation of assay-ready compound plates, washing tips in between plates is often used to contain costs. Because of variable compound characteristics (hydrophobic, potent, etc.) or the sensitivity of the biological assay, careful evaluation of the tip washing method is required. Therefore, whenever new program support is initiated, liquid-handling methods are evaluated for specific chemotype and biological assay combination. The results of an experiment that failed a carryover test are highlighted in **Figure 5** through observed activity in blank DMSO plates. In this experiment, a series of concentration-response curves was generated for potent compounds. The highest concentration of compound was in columns 1 and 11. After compounds were transferred to the assay-ready plates, the tips were washed through multiple cycles of DMSO and water. Using the same tips, a second assay plate was then created by transferring just DMSO. This process was repeated to create a third plate with DMSO. All 3 plates were tested in the biological assay. Higher activity in **Figure 5** is designated by greens with a gradation to reds for no activity. The compound plate showed the appropriate concentration response in the assay. The DMSO plates should show no activity by exhibiting red in every well. As seen in **Figure 5**, the DMSO plates showed activity in some wells, albeit reduced from the original plate. The potential for confounding data is seen in **Table 2**. As indicated by the >50 μM inhibitory concentration value, the wash method adequately cleaned the tips for some chemotypes. However, other chemotypes exhibited potent responses after 1 or even 2 wash cycles. If carryover cannot be removed by increasing the number of cycles of water and DMSO washes or using other compatible solvents such as methanol, then changing tips in between plates is employed.

CONCLUSIONS

Preserving compound integrity and accurately providing compounds are crucial to the drug discovery process. Both of these challenges can ultimately influence drug discovery decisions by either missing or falsely identifying compounds of interest. While maintaining these ideals, CM is also challenged with throughput and capacity constraints. Although instrumentation and automation have helped to address these constraints for liquid transfers, the processing of solid samples is still demanding. The VST process described above goes a long way to address this situation. Compounds of all physical characteristics, including gums and oils, can be handled in an automated manner. However, care needs to be taken in controlling evaporation temperatures and ensuring complete solvent removal.

An additional challenge is establishing the appropriate storage conditions for compounds. Multiple storage options are used to preserve compound integrity while sustaining process

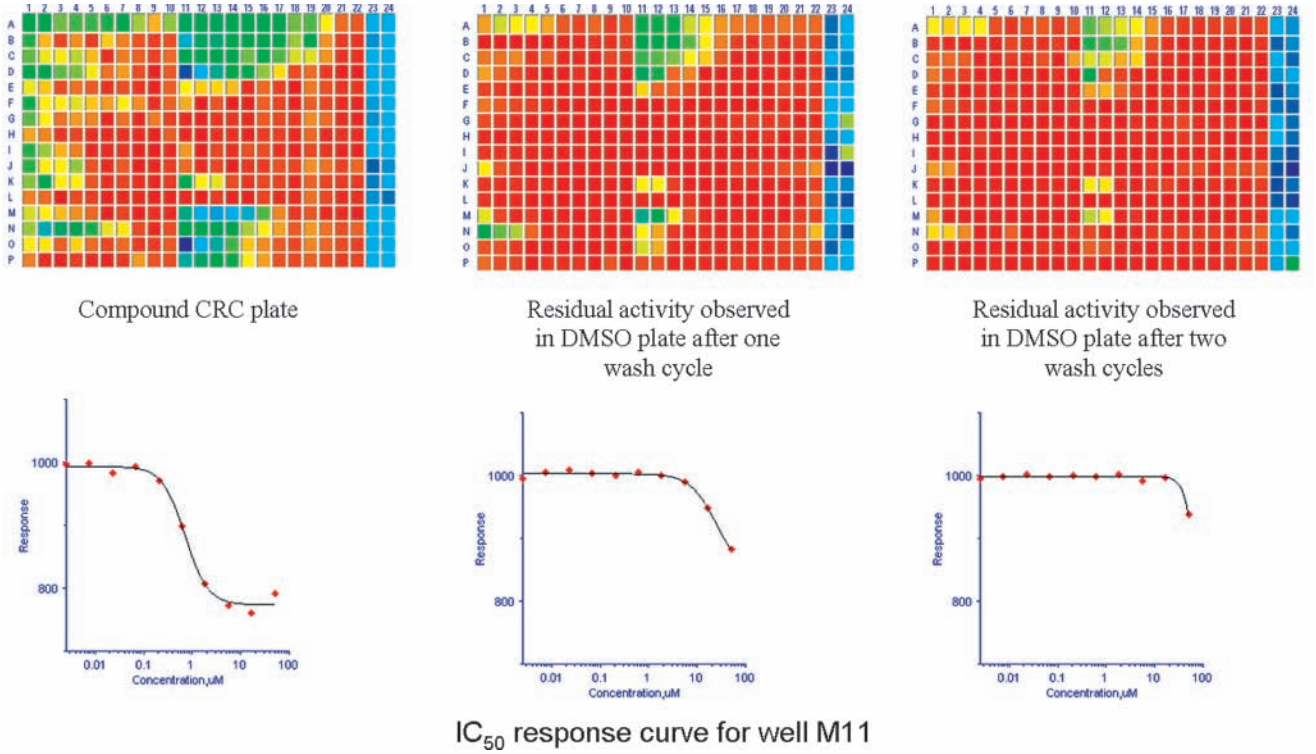


FIG. 5. Example of observed compound carryover from a compound-response curve (CRC) source plate in subsequent DMSO control plates (indicated by green wells).

Table 2. Half Maximal Inhibitory Concentration (IC_{50}) Values (in μM) of Compounds That Exhibited Carryover after Washing Tips with Water and DMSO

	Half Maximal Inhibitory Concentration (IC_{50}) Values (μM)		
Well Number	*Compound Plate*	*DMSO Plate (1 Wash Cycle)*	*DMSO Plate (2 Wash Cycles)*
A11	0.07	3.1	2.9
B11	0.1	2.1	6.8
C11	0.1	2.5	12
D11	2.5	40	>50
M11	1.2	20	>50
O11	10	>50	>50

flows. Storing samples as solids within low-temperature, regulated humidity conditions is optimal for maintaining compound integrity for long periods. Establishing process flows, such as evaporating and reconstituting plates, addressed changing needs caused by assay miniaturization in the hit identification arena. For short periods, room temperature storage conditions can be used for handling liquid inventories when iterative testing is required. Incorporation of methodology to avoid water absorption in DMSO solution, either during storage or processing, contributes toward preserving accurate concentrations of samples. In addition, dependable data generated across multiple assays are achieved by a consistent compound-handling process. This commonality of practice in lead optimization has proved beneficial in maintaining compound integrity.

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