Studies on Repository Compound Stability in DMSO under Various Conditions

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The chemical stability of repository compounds is affected by various environmental conditions during long-term storage. Studies were carried out to evaluate the effects of the following potential causes of instability of compounds in DMSO at a 10-mM concentration: water, oxygen, freeze/thaw cycles, and storage container material. A set of compounds was selected for the study based on structural diversity and functional group representation. Compound concentration was determined with liquid chromatography/ultraviolet spectroscopy/mass spectrometry (LC/UV/MS) analysis relative to an internal standard added to each sample. An accelerated study was conducted, and results demonstrate that most compounds are stable for 15 weeks at 40 °C. Water is more important in causing compound loss than oxygen. The freeze/thaw cycle study was done with freezing at –15 °C and thawing under nitrogen atmosphere at 25 °C. Two methods were used to redissolve compounds after thawing: agitation and repeated aspiration/dispense. The results indicate no significant compound loss after 11 freeze/thaw cycles. Compound recovery was also measured from glass and polypropylene containers for 5 months at room temperature, and no significant difference was found for these 2 types of containers. (*Journal of Biomolecular Screening* 2003:292-304)

Key words: DMSO; repository compound stability; high-throughput screening; compound decomposition; freeze/thaw; liquid chromatography/ultraviolet spectroscopy/mass spectrometry (LC/UV/MS)

INTRODUCTION

COLLECTIONS OF LARGE NUMBERS OF CHEMICAL COMPOUNDS are commonly used in pharmaceutical research. These compounds are typically acquired over the course of many years, added to the collections through purchases and collaborations, the synthetic efforts of a company's own scientists, or the gathering of natural products. They are used for many elements of the research process, including initial screening; hit validation; structure-activity relationship (SAR) studies in lead optimization; absorption, distribution, metabolism, and excretion (ADME) and toxicity evaluations; analytical studies; and technology development experiments. The size and the diversity of compound collections are generally deemed important for the success of the initial lead discovery efforts. Thus, large efforts are devoted to increasing the numbers and the structural diversity of the collection. However, few studies on the purity and stability of the compounds have been reported in the literature, although with the widespread use of combinatorial chemistry, measuring the purity of the library compounds at the time of synthesis has been discussed.[1,2]

In practice, the quality of the repository compounds is very important to drug discovery efforts. Poor sample quality can cause many problems in biological assays. Impurities and decomposition products may result in false positives. A lower than expected concentration of target compounds could cause difficulty with accurate IC_{50} and K_d determinations and even preclude the possibility of finding an important drug lead in primary screening. In addition, unstable compounds, if not handled properly, can cause assay inconsistency due to time-dependent changes in the concentration of target compounds and impurities. It is not uncommon for a large pharmaceutical company to have a repository with as many as a million compounds. Thus, if even 10% to 15% of the collection is made up of poor-quality compounds, it may lead to the waste of hundreds of thousands of dollars per year in primary screening costs. A poor-quality compound can cause even more waste of resources when it is subsequently tested in follow-up assays.

Although these research collections are usually quite dynamic in nature, with many new samples entering them or being depleted each year, many of the compounds remain in a repository for decades. They have traditionally been stored in glass vials in a dry format. As the size of these collections grows into the hundreds of

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thousands and even millions, the need to rapidly weigh and repackage large numbers of the individual samples poses a significant logistics challenge to the repository staff. Also, as chemical and biological detection methods become increasingly sensitive, the amount of compound required per test continues to decrease, often reaching the point where it becomes difficult and time-consuming for technicians to accurately weigh the small (below 1 mg) amounts requested; as a result, they often provide excess samples, resulting in waste and accelerated depletion of the stocks.

Automation can be employed to resolve some of these problems, particularly if the samples are available in a liquid format. It is generally believed that storage in the dry state affords a longer "shelf life" compared to storage in solution. In addition, the solvent itself may catalyze or participate in certain reactions, leading to the degradation of the compounds. However, the use of solvent is generally a necessity for the automated dispensing of large numbers or small amounts of samples. It is relatively easy to automate the transfer of liquids using available high-speed pipetting equipment, and many companies routinely store portions of some or all of their collections in a solvent for this purpose. DMSO is the most common solvent used due to its good solvating ability for compounds, relative chemical inertness, and relatively high boiling and freezing points. The samples are stored either at room temperature or in a frozen state and thawed when needed.

In addition to the storage format, time, temperature, and the structural characteristics of the compound, many other factors may affect repository compound stability. These include humidity, atmosphere (oxygen), repeated freezing and thawing, container materials, salt forms, the presence of impurities, and so forth. Information about the relative importance and actual magnitude of these factors is desirable to select the optimal format and conditions for repository compound storage and handling. Surprisingly, there has been a paucity of reports in the literature on repository compound stability. The first published studies of this type were done by researchers from ComGenex[3,4] who developed Stabex, a systematic method of assessing the stability of repository compounds generated through combinatorial synthesis. In Stabex, the stability is measured experimentally at several elevated temperatures (e.g., from 115 to 190 °C) for selected compounds in a library, and the average Arrhenius parameters are obtained. These parameters are used to predict the expiry rate (shelf life) of the library at a selected storage temperature. The prediction is verified using experimental measurement at some intermediate temperatures (e.g., 75 °C and 100 °C). The authors found that the observed shelf life for the majority of the compounds investigated at an intermediate temperature agreed well with the predictions based on kinetic parameters obtained at elevated temperatures. In 1 case, 8 out of 10 compounds showed good agreement (< 15% difference between prediction and experiment). The authors used the Stabex method to study a small repository of 3000 compounds comprising 300 libraries, and results allowed projections that 96% of the compounds would be stable for 2 years or longer at 25 °C in the solid state. Recently, a number of studies on repository compound stability or purity, either in solid state or in solution, have been reported at scientific meetings.[5-15] Some of these studies focused on specific aspects of the compound stability (e.g., freeze/thaw study).[9] Others relied on qualitative measurement and statistical analysis to draw conclusions.[8,10] Studies on the stability of individual drugs in specific formulations at preclinical and clinical stages have been documented extensively, as required by the Food and Drug Administration (FDA) for drug approval.[16-18] These studies, although not applicable to repository compounds, nonetheless provide useful insights into some aspects of repository compound stability. For example, Stanaszek and Pan[19] reported no significant differences in stability for several common drugs in glass versus plastic syringes.

In this paper, we report stability studies of 644 repository compounds in DMSO at a 10-mM concentration under various conditions based on quantitative liquid chromatography/ultraviolet spectroscopy/mass spectrometry (LC/UV/MS) measurements. The conditions investigated include an accelerated stability study at 40 °C, with atmospheric conditions controlled for the presence and absence of humidity and oxygen, repeated freezing and thawing, and different container materials. Studies on longer term stability at room temperature and –15 °C and an accelerated stability study for compounds containing TFA salt are also under way, the results of which will be published later. Preliminary results of this work were presented at the national meeting of the American Chemical Society (Chicago, 2001)[20] and the post–Drug Discovery Technology 2002 Session on Repository Compound Stability Studies (Boston, 2002).[21]

MATERIALS AND METHODS

Experimental design for compound stability studies

The experimental design of the study was built around an investigation of important factors causative of compound decomposition over time. Important environmental factors include the effect of humidity (water) and oxygen on compound decomposition. Therefore, the design of the experiment was such that these 2 factors could be investigated independently and in conjunction with one another. This was achieved by the construction of atmospheric control chambers, which were filled with pure nitrogen or synthetic "air" (20% oxygen, 80% nitrogen). Furthermore, the samples to be studied were then dissolved in either DMSO or DMSO/H_2O (95:5 v/v). The selection of the concentration of aqueous DMSO was made based on a ^{1}H-NMR analysis of the percentage of water in a historical high-throughput screening (HTS) collection of compounds in DMSO to match the percent absorption seen over a typical handling lifetime of those samples.

Time and temperature are 2 interrelated factors affecting the stability of compounds stored in a liquid format. To gain information rapidly, we began an accelerated study, with all samples stored at 40 °C under their respective environmental conditions. This information is being confirmed by a room-temperature storage set and a frozen storage set created and stored simultaneously with the

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Table 1. Design for Repository Compound Stability Studies in DMSO at a 10-mM Concentration

Accelerated 1	*T (°C)*	*Container*	*T0 Reference*	*DN*	*WN*	*DO*	*WO*	
26 weeks	40	Glass	X	X	X	X	X	
Accelerated 2	*T (°C)*	*Container*	*T0 Reference*	*3 Weeks*	*9 Weeks*	*17 Weeks*	*26 Weeks*	
Dry	40	Glass	X	X	X	X	X	
Wet	40	Glass	X	X	X	X	X	
Long Term	*T (°C)*	*Container*	*T0 Reference*	*3 Months*	*6 Months*	*9 Months*	*. . .*	*24 Months*
RT	22	Glass	X	X	X	X		X
Frozen	–15	Glass	X		X			X
Freeze/thaw	*T (°C)*	*Container*	*Dissolution*	*T0 Reference*	*1 Cycle*	*5 Cycles*	*10 Cycles*	*11 Cycles*
FT	22/–15	Glass	None	X	X	X	X	X
FT-AG	22/–15	Glass	AG	X	X	X	X	
FT-SS	22/–15	Glass	SS	X	X	X	X	
Containers	*T (°C)*	*Container*	*T0 Reference*	*5 Months*	*10 Months*			
Glass	22	Glass	X	X	X			
Plastic	22	Polypropylene	X	X	X			

X = sampling. Wet samples contain 5% water (v/v). DN = dry samples under nitrogen atmosphere; WN = wet samples under nitrogen atmosphere; DO = dry samples under a mixture of 20% oxygen and 80% nitrogen; WO = wet samples under a mixture of 20% oxygen and 80% nitrogen; RT = room temperature; FT = freeze/thaw; AG = agitation dissolution; SS = "suck-and-spit" or dissolution by repeated aspiration and dispensing.

accelerated study set, the results of which can eventually be compared to the accelerated (40 °C) study. It is believed that frozen storage will likely provide a more stable environment for the compounds. However, depending on the format of the storage, repeated freeze/thaw cycles may be required for compound dispensing, and sample stability under such conditions needs to be evaluated. Water absorption and compound precipitation are important practical issues when freezing and thawing samples. We therefore designed a thawing procedure using a dry nitrogen atmosphere and selected 2 methods of compound dissolution after each freeze/thaw cycle: agitation with a vortexing apparatus (AG) and mixing with repeated aspiration and dispensing using a liquid-handling robot ("suck-and-spit" or SS). In addition, the container material in which compounds are to be stored was thought to have potential consequences for sample stability, either from the standpoint of its permeability to atmospheric conditions (water and oxygen) or in the direct interaction of container materials with stored compounds.

The overall experimental design is shown in Table 1. An initial accelerated study at 40 °C (Accelerated 1) was designed to determine the relative importance of humidity and oxygen for compound stability. Another objective was to determine if 26 weeks was an adequate amount of time for the planned subsequent study (Accelerated 2), which would result in enough compound loss to confidently determine rates of declining compound quality. The results of this initial accelerated study indicated that humidity has a far greater impact than oxygen on stability, and so the later experiments were all done under ambient atmosphere without attempting to control for oxygen exposure. We set the criterion of 80% of the test compounds having 80% or more of the initial concentration remaining as acceptable compound retention.

Analytical method development

There are several common analytical techniques for the quantitation of compounds of diverse properties, including nuclear magnetic resonance (NMR) and liquid chromatography coupled with 1 or more detection techniques such as UV (either single wavelength or diode-array detection), MS, and evaporative light-scattering detection (ELSD). NMR spectroscopy was deemed unsuitable for our studies due to its low sensitivity and throughput. Reverse-phase liquid chromatography coupled with UV diode-array detection, MS, and ELSD were all evaluated for a test set of compounds at different concentrations. Of these 3, UV showed a linear response to the test sample concentration over the widest dynamic range. Therefore, LC/UV/MS detection was chosen for this project, combining the good linear dynamic range of UV with the good sensitivity and peak identification capability of MS. The serial dilution of 12 standard test compounds was investigated with internal and external standard techniques. Internal standardization was found to give the most reproducible quantitation results. To determine the standard deviation of this technique, we completed preliminary reproducibility studies. Figure 1 shows the analysis results of replicates over 3 days for an internal standard against a typical target compound. Data were generated by the integration of a target compound versus an internal standard, with 12 replicates

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Table 2. LC/UV/MS Method Validation

Test Compound	*Retention Time (min)*	*Target/IS*	*Wavelength (nm)*	*Test 1: RSD (%)*	*Test 2: RSD (%)*
1	1.59	6.49	230	3	2
2	1.70	7.03	270	7	6
3	2.14	1.96	280	5	2
4	1.64	2.87	275	5	2
5	1.45	0.14	250	6	1
6	2.72	3.99	286	4	1
7	1.74	0.33	270	7	5
8	1.36	1.29	286	5	2
9	2.59	0.59	276	4	2
10	2.00	1.03	260	5	4
11	1.35	4.10	286	5	5
12	3.03	2.23	290	8	1

Twelve target compounds with diverse optimal wavelengths, retention times, and relative peak area versus the internal standard (IS) were mixed with IS compound at 8 different concentrations. Duplicate samples were prepared for each concentration. Two tests were done with 2 months' separation. The relative standard deviation (RSD) values were calculated against a linear fitting of the observed target/IS ratio versus the theoretical concentration ratio. LC/UV/MS = liquid chromatography/ultraviolet/mass spectrometry.

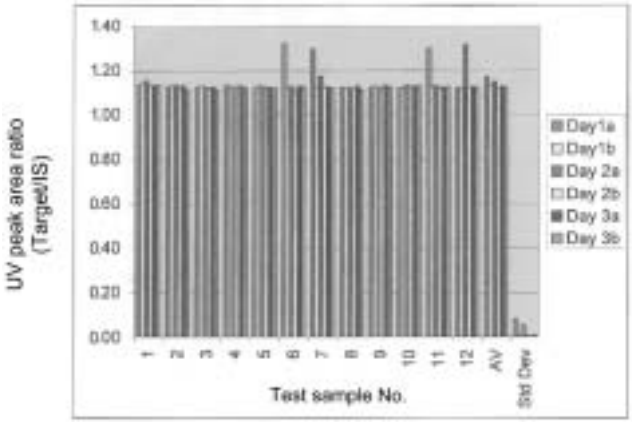

FIG. 1. Reproducibility of the analytical technique. Twelve samples of the same concentration ratio of target versus internal standard were prepared and injected twice daily over 3 days. The *x*-axis represents samples 1 through 12 along with average and standard deviation values. The *y*-axis represents the UV area ratio of the target versus internal standard.

each over 3 days and analysis twice per day. Standard deviation appears quite good for these internal standardization conditions. The internal standard (IS) compound used for the compound stability studies, N-benzoylaminopurine (Fig. 2), was selected based on its early elution (1.1 min, faster than 98% of the compounds selected), good MS signal, and broad absorption band. For quantitation, an optimum wavelength was selected for each compound to balance UV absorption between the IS and the target compound. Quantitation performance of the LC/UV/MS method was validated by analyzing the set of 12 test compounds with diverse optimal wavelengths, retention times, and relative peak area versus the

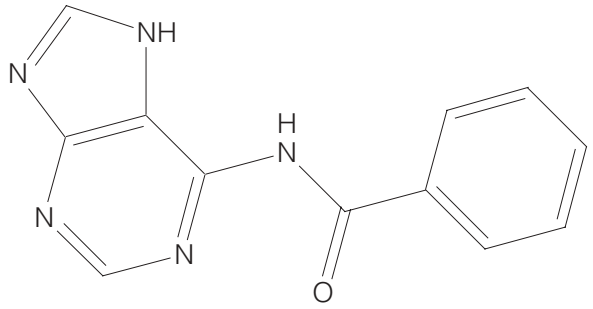

FIG. 2. Internal standard compound (N-benzoylaminopurine).

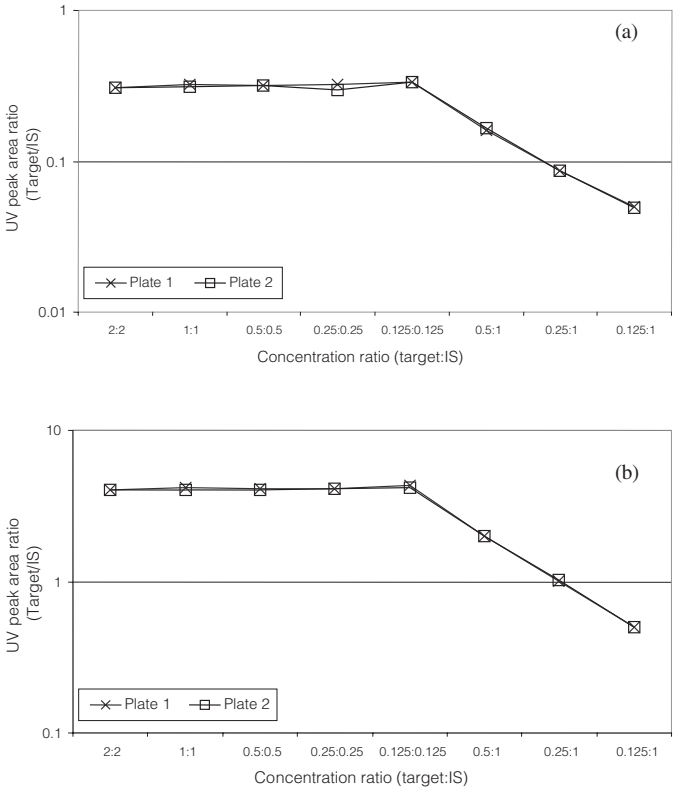

FIG. 3. Selected examples of liquid chromatography/ultraviolet spectroscopy/mass spectrometry (LC/UV/MS) method validation. Target compound was mixed with internal standard, with ratios indicated on the *x*-axis (1:1 = 200 μM target:50 μM IS), and duplicate samples were prepared in 2 different plates for each compound. Data shown are for test compounds 7 (**a**) and 11 (**b**).

IS. The results are shown in Table 2 for 2 repeated analyses with 2 months of separation. Figure 3 shows plots for 2 representative compounds. The concentrations prepared in the test samples cover cases in which both the test compounds and IS are higher and lower than normal. These different concentrations were used to test the

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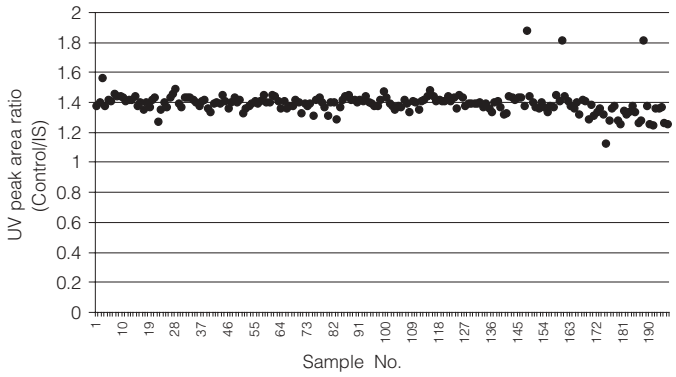

FIG. 4. Observed UV peak area ratio of the control compound versus internal standard (IS) compound. The data represent 7 consecutive sets of compound stability samples. There are 4 control samples in each 96-well plate and 7 plates in each compound stability study set. See text for details.

possibility of injection volume variations and concentration changes due to solvent evaporation. The results indicate no effect on quantitation when the concentrations of test and IS compounds are both changed to up to 2 times higher or down to 8 times lower than the normal concentration. These results also establish that a good linear dynamic range can be obtained at target/IS ratios up to 10 and down to 0.03. We determined this performance to be satisfactory for the objectives of the compound stability studies.

Four samples containing 1 control compound were included in each 96-well plate containing stability study compounds to assess the uncertainty for compound dispensing, sample preparation, and analysis. The control compound was initially dispensed into separate plates from the stability study samples with a similar dispensing procedure. Four control compound samples were then added to each plate containing 92 test compounds after each stability study and before addition of the IS/MeOH solution for LC/UV/MS quantitation. Figure 4 shows the observed UV peak area ratio of the control compound versus IS for 7 consecutive sets of stability study samples. The data reflect the combined error of compound dispensing, IS/MeOH solution preparation, IS sample addition, mixing, and LC/UV/MS analysis.

Compound selection

A total of 1500 compounds were initially selected to represent the structural diversity of the repository compounds at Abbott Laboratories. The compounds were chosen based on standard clustering analyses. To ensure the identity and purity of all compounds included in the study, we conducted a survey analysis with LC/UV/MS. Of the compounds, 981 were selected based on the following criteria: 1) good UV absorption, 2) chromatography retention time not overlapping with void or IS, 3) MS data confirming peak identity, and 4) samples with an impurity less than 10% as estimated by ELSD and UV at 220 nm. Functional group representation was used as the criterion in the next step of the selection process. The 981 compounds were analyzed to identify the distribution of 25 common functional groups. Then, 644 compounds (7×92) were selected for the compound stability study based on the following criteria: 1) at least 25% of any group present in > 10 of the original 1500 compounds was not excluded, and 2) no salt form present in > 5% of the list of 1500 was biased more than 25% either by inclusion or exclusion.

Compound preparation

High-performance liquid chromatography (HPLC) grade DMSO, MeOH, and CH_3CN were used unless otherwise noted. Individual powder samples were dissolved in fresh DMSO to 10 mM in polypropylene 96–deep well plates (Marsh Bio Products, Rochester, NY). The solutions were shaken overnight with a Model 3520 ORBIT shaker (Lab-Line, Melrose Park, IL). Then, 10 µL each of the solutions was dispensed to multiple destination plates using a Zymark (Hopkinton, MA) SciClone liquid-handling robot with a 96-well parallel transfer. The destination plates were 96–deep well plates with v-bottom glass inserts of 450-µL capacity (Webseal, Chromacol, Trumbull, CT).

The destination plates containing 10 µL of each compound were sealed with adhesive sealing foil (Beckman Coulter, Fullerton, CA) and stored under appropriate conditions designed for the specific stability tests. Wet samples were prepared by dissolving compounds in DMSO containing 5% H_2O (v/v) and were dispensed with a similar procedure as the water-free DMSO samples described above. For the initial accelerated study (Accelerated 1), the same procedure was used but with a Packard Multiprobe (Model 204DT, Packard, Meriden, CT) automated pipettor used for compound distribution instead of a Zymark SciClone.

For the freeze/thaw study, 200-µL water-free DMSO solutions of compounds were dispensed into 96–deep well glass plates consisting of 96 individual round-bottom glass tubes of 1.1-mL capacity (Webseal, Chromacol). The plates were sealed with silicon rubber mat (Webseal, Chromacol). For the plastic versus glass container study, the glass containers were the same type of plates used in the freeze/thaw study and were sealed similarly. The plastic containers used were round-bottom polypropylene 96–deep well plates (Marsh Bio Products) and were sealed with adhesive foil (Beckman Coulter). Then, 100-µL water-free DMSO solutions of compounds were dispensed to each plate for the glass versus plastic container study.

Stability testing

Accelerated stability study procedure. Samples in sealed microplates were placed in sealed acrylic chambers ($8.5 \times 6.5 \times 4.5$ inches) under ambient atmosphere and humidity. The chambers were stored in an oven (Napco 6001, Precision Scientific, Winchester, VA) set at 40 °C. The temperature of the ovens was monitored periodically. Temperature readings at different locations in-

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side the ovens were taken prior to the start of the experiments, and no variation beyond ±1 °C was found. The acrylic chambers were removed from the ovens and opened at predetermined time intervals, and the concentration remaining in each sample well was determined for the target compounds. In the initial accelerated study under 4 different conditions (Accelerated 1), the chambers were filled with appropriate gas or gas mixture at the beginning and were purged with the same gas or gas mixture once every week at 10 psi. The contents of each chamber (N/O ratio) were monitored weekly. Drying agent was placed in the chambers containing the samples in water-free DMSO.

Freeze/thaw study procedure. Samples in sealed microplates were stored in a –15 °C freezer for a week before the first freeze/thaw cycle. Thawing was done under a gentle flow of nitrogen for 5 h in an oven (Model 1400E, Sheldon Manufacturing, Cornelius, OR) maintained at 25 °C. The oven was purged with dry nitrogen before plates were transferred into it. The plates were inspected visually for precipitation. After thawing, 1 set of plates (AG plates; see Table 1) was agitated using a vortex stirrer (Multi-tube Vortexer, VWR Scientific Products) for 5 min, and another set (SS plates; see Table 1) was mixed using a SciClone pipettor with 20 repeated aspiration and dispense cycles of 50 µL of the content. After these treatments, the plates were returned to the –15 °C freezer for the next freeze/thaw cycle. Sampling was done after 1, 5, 10, and 11 freeze/thaw cycles. The sampling method was similar to the procedure of initial sample dispensing for the accelerated stability testing. Then, each 10-µL sample was transferred to 96–deep well plates with v-bottom glass inserts of 450-µL capacity (Webseal, Chromacol) with a SciClone pipettor. Each freeze/thaw cycle required one 24-h period with the exception of the 11th freeze/thaw, which was done 5 weeks after the 10th freeze/thaw.

Glass versus plastic container study procedure. Samples in sealed microplates were stored in a temperature-controlled room at 22 ± 2 °C at ambient humidity and atmosphere. The content of the plates was sampled at predetermined time periods for quantitation using LC/UV/MS. The method of sampling is similar to that of the freeze/thaw samples. Then, each 10-µL sample was transferred to 96–deep well plates with v-bottom glass inserts of 450-µL capacity (Webseal, Chromacol) with a SciClone pipettor.

Sample preparation for LC/UV/MS analysis

To each well of the 7 plates of samples for each stability test data point, 450 µL of 50 µM IS compound (N-benzoylaminopurine, Lancaster, Pelham, NH) in MeOH was added using a SciClone pipettor. The samples were mixed by 2 aspiration/dispensing operations. The plates were stored at –50 °C for 3 days. Then, 2 additional mixings were done, and 100-µL of the content was transferred to a v-bottom polypropylene 96-well plate (Orochem, Westmont, IL) for LC/UV/MS analysis. The remainder of the samples was sealed with adhesive foil (Beckman Coulter) and stored at –50 °C. The IS/MeOH solution was prepared fresh every time a set of sample plates was prepared for LC/UV/MS analysis. In the initial accelerated study (Accelerated 1), a Packard 204DT Multiprobe automated pipettor was used for sample preparation with an otherwise similar procedure.

LC/UV/MS analysis

For sample analysis, a Waters (Milford, MA) ZQ single quadrupole mass spectrometer, a Waters model 2790 separations module, and a Waters model 996 photodiode-array detector were used. Reverse-phase liquid chromatography analysis was done with a YMC-AQ 2 × 50 mm column (120 Å, 5-µm C18) from Waters, and several columns of the same lot were purchased to minimize column-to-column variation. The column was heated to 35 °C. The analysis was done with a 10-µL injection at a 1.0-mL/min flow rate. The gradient method used was as follows: 10% B for 1.1 min, from 10% B to 100% B in 3 min, hold at 100% B for 0.7 min, and return to 10% B in 0.2 min (A: 0.1% TFA in water; B: CH_3CN) with a total runtime of 6 min. The 1.0-mL/min flow from the column was split 50:50 for UV and MS analyses. In a typical LC/UV/MS analysis, 4 plates of samples were loaded in the Waters HT autosampler, and another 3 to 4 plates of samples were loaded after completion of the first set, which required 2½ days. The mass spectrometer was scanned from m/z 120 to 1000 at 1 sec/scan. Only positive-ion atmospheric pressure chemical ionization (APCI) spectra were acquired for each sample. Diode-array detection was done from 210 to 350 nm.

Data processing

The UV and MS data were processed with Openlynx software (Waters) with predetermined parameters (m/z values for protonated and sodiated species and UV wavelength) for each compound. UV data (retention time and peak area) were converted to ASCII files and transferred to an Oracle database. Target and IS peaks were assigned based on a lookup table, and the target-to-IS ratio was calculated for each compound. Visual inspections and corrections of peak assignments were done with an in-house written software application based on a Microsoft Excel Visual Basic macro. Results were also inspected using the Waters OpenLynx browser for 1 plate at a time and an in-house written chromatogram data browser for 1 compound at a time in multiple tests. The concentration of each compound was calculated by dividing the observed target/IS value for the test with that of the T0 reference. The T0 reference was obtained before compound stability testing and was the average of duplicates of samples independently prepared and analyzed.

RESULTS AND DISCUSSION

Initial accelerated studies

An accelerated study at 40 °C was conducted over the span of 26 weeks to assess the relative importance of humidity and oxygen on compound stability. The conditions used were dry DMSO under nitrogen atmosphere (DN), wet DMSO (containing 5% water,

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Table 3. Concentration of 644 Compounds after Treatment at 40 °C for 26 Weeks under 4 Different Conditions (Accelerated 1)

Condition	*DN*	*DO*	*WN*	*WO*
Average	.74	.74	.71	.71
Median	.86	.87	.84	.82
80% remaining[a]	57	60	53	53

Concentration values are normalized to that of the T0 reference. DN = dry samples under nitrogen atmosphere; WN = wet samples (5% water, v/v) under nitrogen atmosphere; DO = dry samples under a mixture of 20% oxygen and 80% nitrogen; WO = wet samples (5% water, v/v) under a mixture of 20% oxygen and 80% nitrogen.

a. Percentage of compounds with 80% or more of initial concentration remaining.

v/v) under nitrogen atmosphere (WN), dry DMSO under a mixture of 20% oxygen and 80% nitrogen (DO), and wet DMSO (containing 5% water, v/v) under a mixture of 20% oxygen and 80% nitrogen (WO). The condition of 5% water in DMSO was selected to simulate water absorption by DMSO when stored and handled in ambient humidity conditions. The mixture of 20% oxygen and 80% nitrogen was used to simulate the composition of ambient atmosphere. The data at the 6-month time point are given in Table 3. The differences between the oxygen-free and oxygen samples are much smaller than the differences between the dry and wet samples. These results indicate that humidity is by far a more important factor in affecting compound stability. On the basis of these results, we designed the subsequent compound stability studies with ambient atmosphere without active control of the oxygen content (Table 1).

Accelerated stability studies

As shown in Table 1, the compounds in the Accelerated 2 studies were analyzed at 5 time points: 0, 3, 9, 17, and 26 weeks. Figure 5 shows the overall data for each time point. One can clearly see a gradual decrease in the concentration of the target compounds for both dry and wet samples. Figure 6 shows concentration change over time for selected compounds. The majority of the compounds tested are fairly stable, showing little or no concentration reduction in either dry or wet DMSO at 40 °C for 26 weeks. Some showed various degrees of reduction in concentration, with wet samples showing more loss than dry samples. Figure 7 shows an example of UV chromatograms for different time points when the target compound degraded to form products. The presence of water in the wet DMSO samples clearly shows a detrimental effect on compound stability (Fig. 8). A small fraction of the compounds showed a concentration decrease shortly after 5% water was added to the samples. The compounds that are less stable in wet DMSO may be those that are hydrolytically more labile or those that are of lower solubility in wet DMSO. The presence of water in the DMSO samples may induce precipitation for those compounds with low aqueous solubility and may also promote certain reactions, giving rise to degradation products. If compound degradation is important, we may expect to see formation of new peaks in the UV chromatograms in addition to those observed in dry samples for these compounds. We observed, on average, less than a 10% increase in other peak areas in the UV chromatograms for these compounds in wet DMSO T0 reference samples compared with dry DMSO T0 reference samples, while there was a 40% decrease in target compound peak area in wet DMSO T0 reference samples (data now shown). So sample precipitation is likely an important factor. At 15 weeks of storage time, the percentage of samples having 80% or more of the initial concentration in dry DMSO is just above 80%, meeting our criterion of compound retention, while in wet DMSO, the retention is 6% lower and does not meet the criterion of compound retention. The numerical results of the acceler-

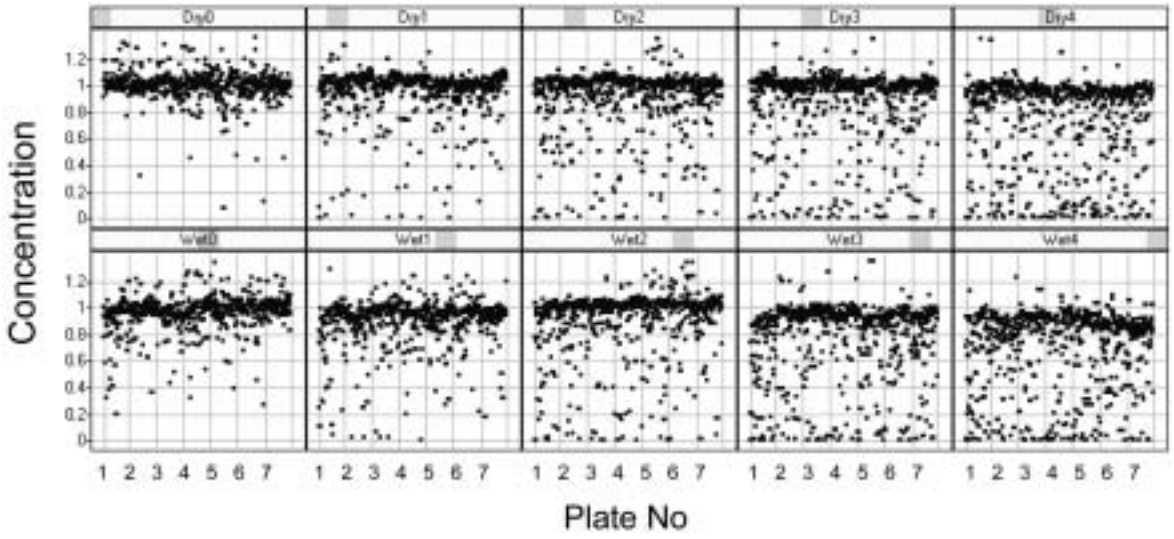

FIG. 5. Concentration for each time point of the accelerated stability study. The top panel shows results for dry samples at time points 0, 1, 2, 3, and 4, and the bottom panel shows results for wet samples at time points 0, 1, 2, 3 and 4. Time point 0, 1, 2, 3, and 4 samples were analyzed at 0, 3, 9, 17, and 26 weeks of storage, respectively.

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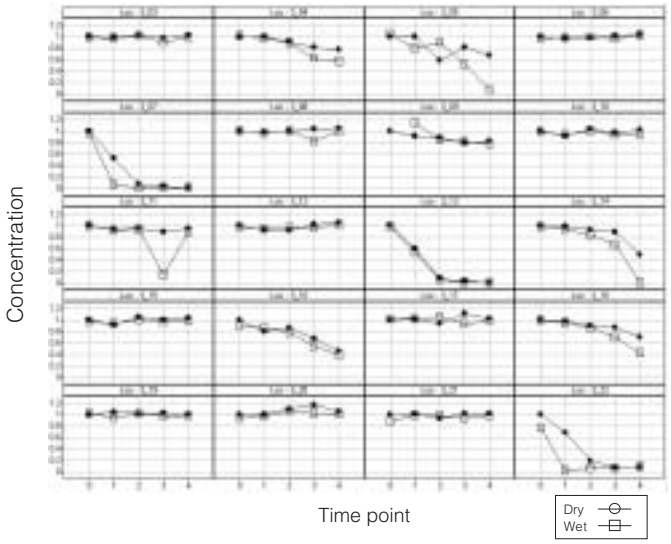

FIG. 6. Comparison of concentration for dry and wet samples for selected compounds. The title bar in each trellis plot indicates sample location. The plots show results for compounds in plate 3, well 3 through plate 3, well 22.

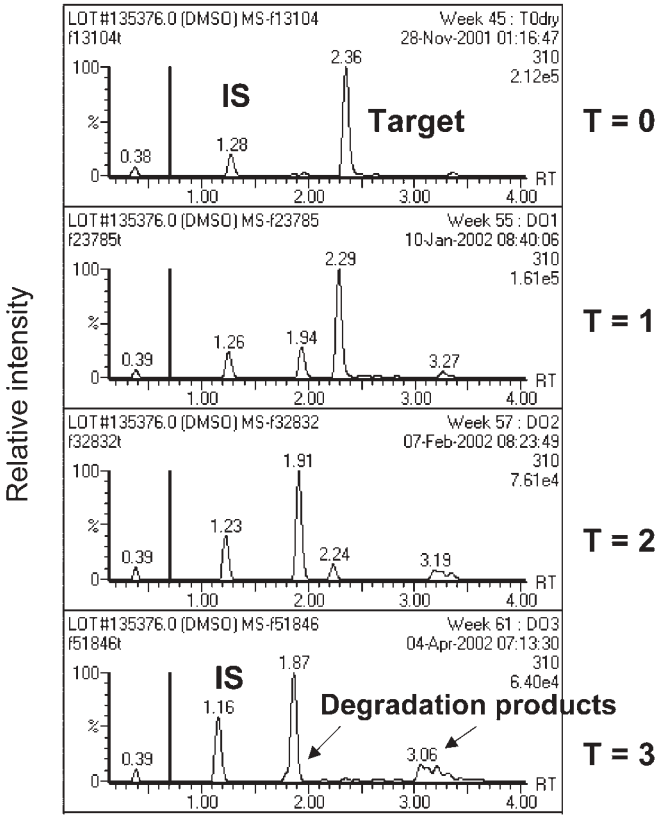

FIG. 7. UV chromatogram for a compound showing concentration change for the target and degradation products after treatment at 40 °C in water-free DMSO. Time point 0, 1, 2, and 3 samples were analyzed at 0, 3, 7, and 17 weeks of storage at 40 °C, respectively.

Table 4. Concentration of 644 Compounds in the Accelerated Stability Study (Accelerated 2) at Different Time Points

Time Point	*T0*	*T1*	*T2*	*T3*	*T4*
Time (weeks)	0	3	9	17	26
Dry					
Average	1.02	0.95	0.90	0.86	0.78
Median	1.00	1.01	0.99	0.98	0.96
80% remaining[a]	96	89	83	78	66
Wet					
Average	1.02	0.89	0.89	0.76	0.74
Median	1.01	0.94	0.99	0.90	0.88
80% remaining[a]	91	84	80	66	63

Concentration values are normalized to that of the T0 reference.

a. Percentage of compounds with 80% or more of initial concentration remaining.

ated stability study are shown in Table 4. With a simple kinetic model that assumes a 2-fold increase in the rate of decomposition with every 10 °C increase in temperature, the dry DMSO sample stability at 40 °C can be projected to an estimated 1 year of acceptable stability at room temperature. The concentration remaining values after 26 weeks of storage at 40 °C are all higher in the Accelerated 2 study than those in the Accelerated 1 study. This difference may be caused by the slight different chromatography conditions used in the analysis of T0 reference samples for the Accelerated 1 study than those used for all other samples in this work. This difference in chromatographic conditions may cause an upward shift of the target/IS values for the T0 reference samples in Accelerated 1 but will not affect the relative values for the samples after 26 weeks of treatment at 40 °C under 4 different conditions.

Freeze/thaw study results

Figures 9 and 10 and Table 5 show the results of the freeze/thaw study. Compounds were sampled after the 5th freeze/thaw cycle, but the samples were not analyzed. Overall, there is little decrease in the compound concentration, observed after the 11th freeze/thaw cycle. This result is quite similar to that of a recent report on a freeze/thaw study with 160 repository compounds at 2 mM in DMSO, in which the authors reported negligible loss of compounds after 25 cycles of freeze/thaw compared to compound loss from storage at 4 °C for 3 months.[6] However, quite different results were reported recently from freeze/thaw studies with 320 compounds at a 20-mM concentration in DMSO, in which the authors observed more than 10% compound loss after 10 freeze/thaw cycles.[9] It is possible that these results reflect the difference in the concentration of samples used in each experiment. The higher the compound concentration, the more likely that compound precipitation will take place during freeze/thaw. In our study, there is no apparent difference between the freeze/thaw results and results from added dissolution (e.g., the agitation and the repeated aspiration/dispensing methods). This is understandable because the compound loss overall from freeze/thaw is minimal and so is com-

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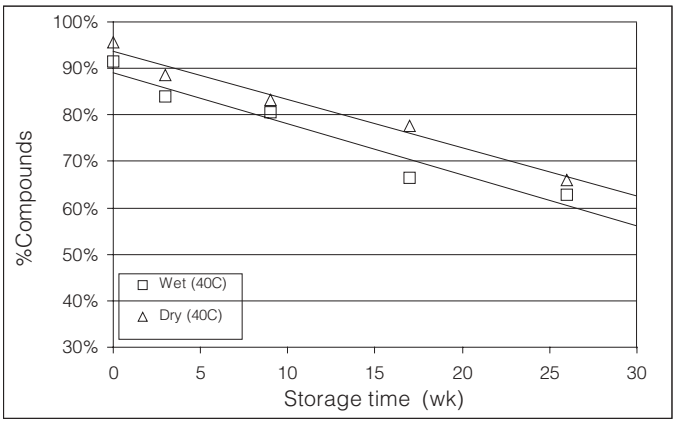

FIG. 8. Plot of percentage of compounds with 80% or more of the initial concentration remaining versus storage time in DMSO at 40 °C. The lines represent linear fitting of the data.

pound precipitation. We have visually checked the bottom of the (glass) vials of the plates and observed precipitation of 2% of the compounds after 1 freeze/thaw cycle, 3% after 10 cycles, and 3% after the 11th cycle. In general, there is a good correlation between the visually observed precipitation of compounds and the decrease in concentration after freeze/thaw, indicating that precipitation is an important factor for compound loss during freeze/thaw. As shown in Figure 11, only 11% of the compounds that exhibited no observed precipitation had a remaining concentration less than 80% of T0 versus 55% remaining of the compounds for which precipitation was observed. The ratio of other peak area/target peak area did not increase through 11 cycles of freeze/thaw, indicating that degradation of compounds is not important to compound loss in the freeze/thaw process.

Table 5. Concentration for Freeze/Thaw Samples

Experiment	*FT*	*FT*	*FT-SS*	*FT-AG*	*FT*	*FT-SS*	*FT-AG*	*FT*
FT cycle	0	1	1	1	10	10	10	11
Average	0.98	0.96	0.98	0.97	0.96	0.98	0.97	0.97
Median	1.00	0.99	1.00	1.01	1.00	1.01	1.01	1.01
80% remaining[a]	94	91	93	89	88	93	89	86

Concentration values are normalized to that of the T0 reference. FT = freeze/thaw; AG = agitation dissolution; SS = "suck-and-spit" or dissolution by repeated aspiration and dispensing.

a. Percentage of compounds with 80% or more of initial concentration remaining.

Glass versus plastic container study

In principle, container materials can affect compound stability and recovery after storage in several different ways. There is the potential for compound to be absorbed into or adsorbed onto the container wall. Because plastics are generally more porous than glass, it is conceivable that plastic containers may cause more compound loss due to absorption than glass. The high porosity of polypropylene can result in the permeation of oxygen and water from the surrounding environment. Also, the surface of the containers may catalyze the degradation of compounds (e.g., residual silanol groups on the surface of certain glass containers). In this regard, plastic containers may be more inert for compounds. In addition, plastic containers may contain plasticizer and other additives, which can be extracted by DMSO. In this study, we selected a type of polypropylene container that contains a low amount of

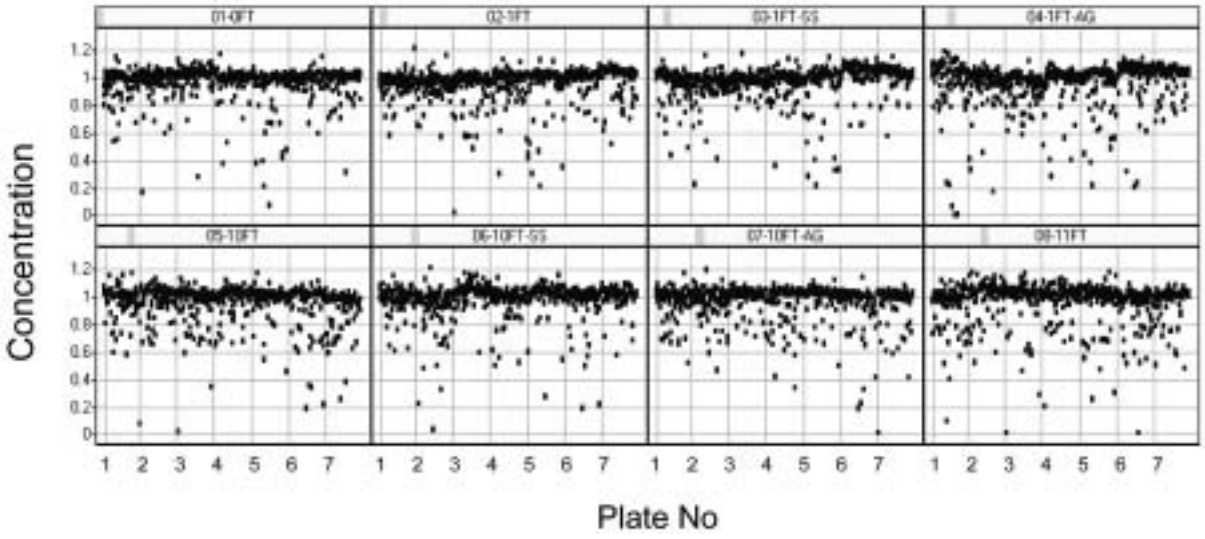

FIG. 9. Concentration for freeze/thaw studies. The top panel shows results for T0 reference (0FT), 1 cycle of freeze/thaw (1FT), 1 cycle of freeze/thaw with "suck-and-spit" dissolution (1FT-SS), and 1 cycle of freeze/thaw with agitation dissolution (1FT-AG), respectively. The bottom panel shows results for 10 cycles (10FT), 10 cycles with "suck-and-spit" dissolution (10FT-SS), 10 cycles with agitation dissolution (10FT-AG), and 11 cycles (11FT) of freeze/thaw, respectively.

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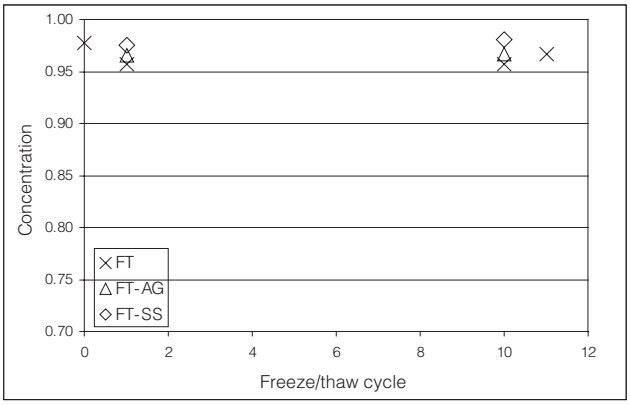

FIG. 10. Average concentration for freeze/thaw samples. FT = freeze/thaw; AG = agitation dissolution; SS = "suck-and-spit" or dissolution by repeated aspiration and dispensing.

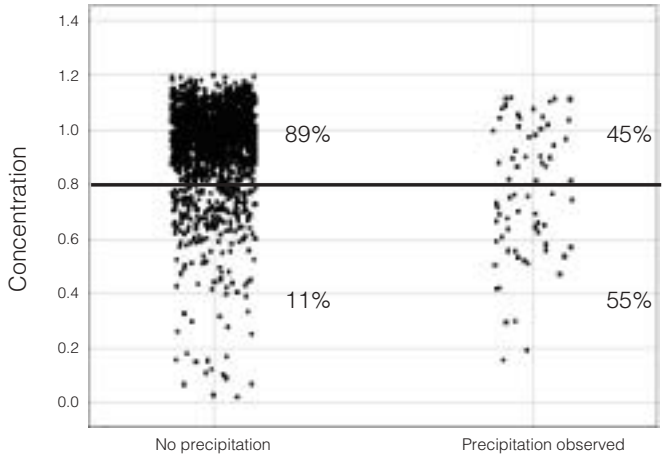

FIG. 11. Correlation between compound concentration in freeze/thaw (average of 10th and 11th cycles) and observed compound precipitation during freeze/thaw. The plots show distribution of concentration values for those compounds for which precipitation was observed (right) or not observed (left) during freeze/thaw.

Table 6. Concentration for Glass/Plastic Container Samples and Available Data for Long-Term Room Temperature (RT) Storage Samples

Experiment	*Glass*	*Plastic*	*RT*	*RT*
Storage time (months)	5	5	3	6
Average	.93	.95	.91	.88
Median	.98	.99	.98	.96
80% remaining[a]	87	90	85	80

All samples were stored at room temperature. Concentration values are normalized to that of the T0 reference.

a. Percentage of compounds with 80% or more of initial concentration remaining.

extractable material according to the manufacturer's specifications. There are previous reports of studies on the stability of marketed drugs in glass versus plastic syringes, and the results indicate no detectable difference in the 2 types of containers.[19] In this project, after 5 months of storage at room temperature, we have observed very little difference in the concentration of these 644 compounds studied in the 2 types of containers (Fig. 12 and Table 6). ^{1}H-NMR measurements indicated the presence of an extractable material from the polypropylene container at the level of 20 µM (total hydrogen atom integration) when 500 µL DMSO was stored in the container for 5 months. Figure 12 and Table 6 also contain available data from the long-term stability study at room temperature for 3 and 6 months for comparison.

Early results of the long-term room-temperature storage study and temperature dependence of compound loss

The concurrent long-term studies for room-temperature and frozen storage are under way, and we have preliminary data for room-temperature samples at 3- and 6-month time points. The results can be seen in Figure 12 and Table 6. Figure 13 combines the room-temperature data and the accelerated study data obtained at 40 °C. It is observed that the rate of compound loss for room-temperature samples (the linear fit line in Fig. 13) shows an ap-

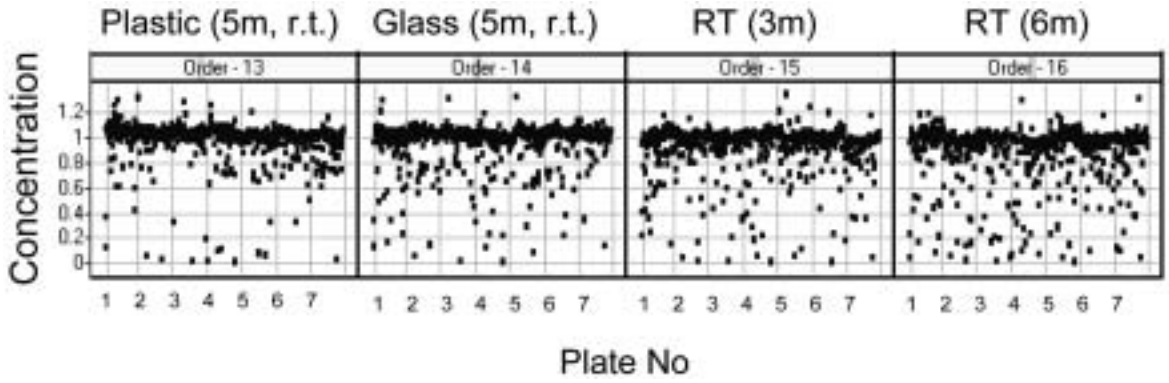

FIG. 12. Concentration for glass, plastic, and room-temperature (RT) storage samples.

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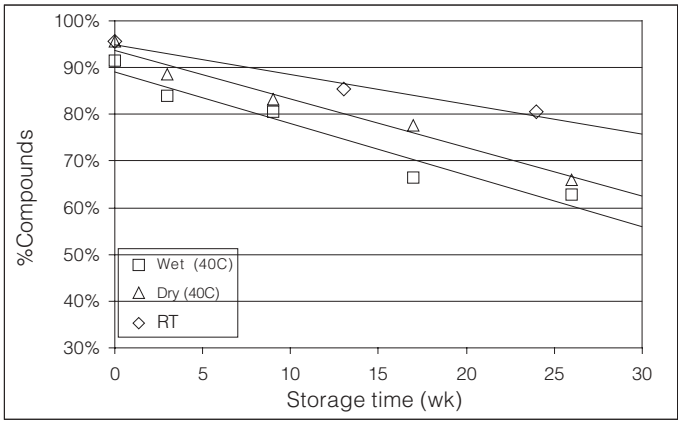

FIG. 13. Percentage of compounds with 80% or more of the initial concentration remaining versus storage time for the accelerated study and for the longer term room-temperature (RT) storage study. The lines represent linear fitting of the data.

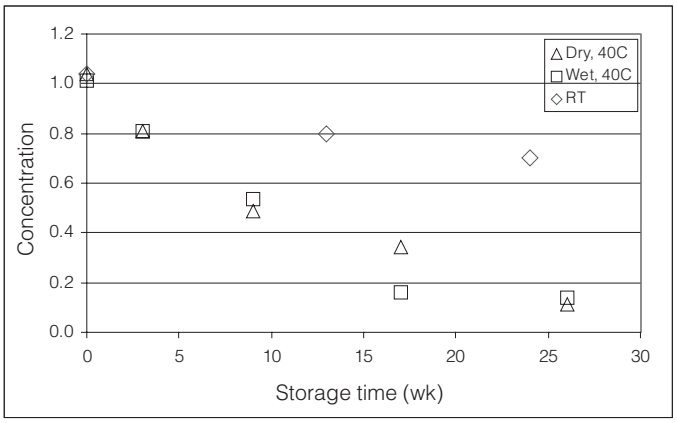

FIG. 14. Average concentration for 52 unstable compounds for which new UV peaks were observed after storage. RT = room temperature.

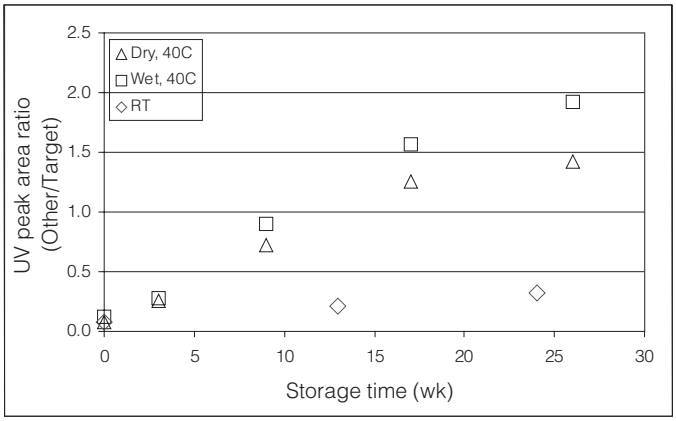

FIG. 15. Observed UV peak area ratio of other peaks versus the target peak for 52 unstable compounds for which new UV peaks were observed after storage. RT = room temperature.

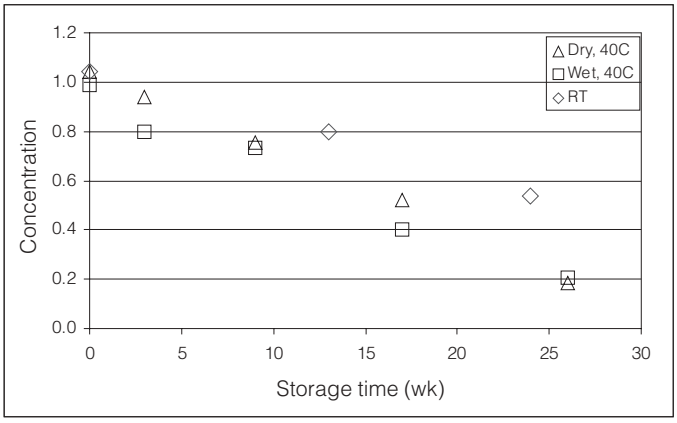

FIG. 16. Average concentration for 45 unstable compounds for which no new UV peaks were observed after storage. RT = room temperature.

proximate factor of 2 times slower than the accelerated samples at 40 °C. This difference is considerably smaller than expected based on the Arrhenius kinetics with reasonable activation energies for thermal degradation.[22] It is generally assumed that there would be a factor of a 2 to 4 increase in the degradation rate with every 10 °C increase in temperature.[22-24] However, when the 52 unstable compounds with new UV peaks are examined (Figs. 14-15), the rate of target compound loss is in fact much more than a factor of 2 faster at 40 °C than at 22 °C. Similar analysis of 45 unstable compounds without new UV peaks shows a smaller difference in the rate of compound loss between 22 °C and 40 °C experiments (Fig. 16). Possibly, for some of these latter unstable compounds that did not give new UV peaks, the concentration decrease is due to processes other than thermal degradation, such as precipitation or absorption. These processes may not abide by the same Arrhenius kinetics as does the thermal degradation process. These observations point out the limitation of projecting data obtained at an elevated temperature to predict compound stability at lower temperatures. Some processes may have weak temperature dependence, and different mechanisms of compound loss may be important at different temperatures. The preliminary results of the long-term room-temperature storage study indicate a shelf life of approximately half a year for these 644 compounds, with the criterion of 80% of compounds having 80% or more of the initial concentration remaining as the acceptable compound retention.

CONCLUSIONS

The stability in DMSO of a set of 644 compounds selected for diversity from the repository collection at Abbott Laboratories has been studied. Each sample was prepared at a concentration of 10

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mM, as was required for HTS, and studied under a variety of storage conditions. Stability factors investigated included the effects of oxygen, water, temperature and time, storage container material, and the effect of multiple freeze/thaw cycles for frozen samples. The analytical technique employed for these studies was LC/UV/MS with UV as the quantitation method. The first experiment undertaken was an accelerated 40 °C study designed to determine the effects of oxygen and water on stability. The result from this indicated that water had a greater deleterious effect on stability than did oxygen. It was found that the majority of compounds stored in water-free DMSO for 15 weeks retained good stability, which can be projected to 1 year at room temperature, assuming the decomposition rate increases by a factor of 2 for every 10-degree temperature increase. Preliminary results on the long-term storage study indicated certain limitations of this projection and an approximate half-year shelf life for room-temperature storage. Samples containing 5% water in DMSO are less stable than water-free DMSO, and thus humidity control is very important for maintaining the integrity of repository compounds. Samples stored in frozen DMSO at a 10-mM concentration were subjected to multiple freeze/thaw cycles. The results indicated that no significant freeze/thaw effects on compound integrity were observed after 11 cycles at this concentration. Therefore, neither decomposition nor precipitation appeared to be a problem. The effect of glass versus plastic container materials was also investigated. The results from this investigation indicated that no appreciable difference in compound recovery during 5 months of sample storage was observed between the two. Polypropylene containers also showed no appreciable amount of extractable material, and as they are generally easier to work with in automated systems, they are therefore quite appropriate for storing repository compounds in DMSO.

In the course of this study, analytical and data-processing methods were developed for quantitative analysis of test samples. These and other current studies on repository compound stability should provide important data for the selection of appropriate conditions and formats for sample storage and handling. The information should also be useful for scientists who work with diverse organic compounds and are concerned about stability and proper handling of compounds. In addition, structural features that make compounds prone to degradation may be important considerations in the selection of compounds that are suitable for drug discovery and development. Ongoing data-mining experiments are being carried out to assess in depth what structural correlations can be determined between stability and structure. We are also conducting studies on the effect of TFA salt on repository compound stability as more and more compounds are prepared by high-throughput organic synthesis (HTOS) where TFA is frequently used in compound purification by LC/MS. Results of these studies will be published separately.

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