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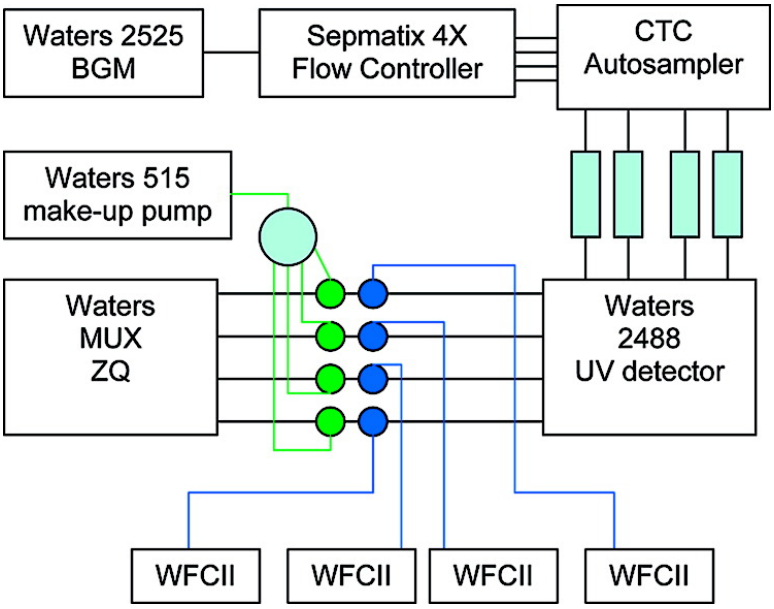

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Purifying the Masses: Integrating Prepurification Quality Control, High-Throughput LC/MS Purification, and Compound Plating To Feed High-Throughput Screening

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In this paper we report using a parallel, four-channel HPLC/MUX/MS purification system, the Purification Factory, to purify thousands of compounds destined for high-throughput screening in a single month. The maximum sample throughput during this 20-workday month was 704 samples/day. Since this purification throughput exceeded the postpurification sample and data handling capabilities provided by commercial solutions, a custom-integrated solution was designed to address these shortcomings. In this paper we detail the key improvements in automation, solvent handling, and sample handling logistics implemented to sustain a mean throughput of 528 samples/day over a multimonth time period.

Introduction

Many pharmaceutical and biotechnology companies possess the high-throughput capability of routinely performing hundreds of thousands or millions of assays per day.[1,2] Ultimately, the quality of the data obtained in a high-throughput (HT) screen is dependent on the robustness of the screen, on the appropriate statistical treatment of the data,[3] and on the quality of the compounds assayed.[4−7] Prior to the development and implementation of high-throughput purification tools, unpurified reaction products were often screened in high-throughput screening (HTS) assays, with the identity and purity of the compound assessed only after the screening. [8,9] Complete analysis of all compounds in a library has been, until recently,[10−12] only practical for small libraries, so the quality of large combinatorial libraries often was determined by analyzing a subset of the library compounds through analysis of a cross-section[13] or of a statistical sampling[14] of the compounds. These strategies predict such properties as yield and purity of library compounds indirectly by inference. In subsequent analysis of compound activity such as structure−activity relationship models, these properties can be taken into account.

Before the advent of high-throughput purification schemes, the quality of compound libraries produced was determined by the successfulness of the chemistry. Difficult chemistries are often needed to access compound libraries that feature activity and druglike properties, and the net result can be a library with a higher incidence of impure compounds. Since the likelihood of encountering false positives and false negatives is reduced by assaying pure compounds,[15,16] high-throughput purification became a necessity as the complexity of HT synthesis products increased. Although classical liquid−liquid[17] and other[18−22] extraction techniques done in parallel could improve the quality of compounds, chromatographic techniques typically provide pure compounds more reliably.[15,18,23−25] UV purification was, and still is, successful in purifying large numbers of compounds each day;[26,27] however, the increased specificity of mass-directed purification[28] permitted purification without prepurification analysis of each sample and provided fractions expected to contain the desired compound (on the basis of *m*/*z*).

Since many research programs depend on the production of large numbers of compounds, the reported throughput of 192 compounds/day achievable with a single-channel LC/MS system proved insufficient, and drove innovations that doubled the throughput to 384 samples using a custom, two-channel, mass-directed LC/MS purification system.[29,30] Soon thereafter, purification throughput was doubled again to 768 compounds/day with the introduction of a vendor-supported,[31] four-channel LC/MS system with a multiplexed source.[32]

Although two- and four-channel LC/MS systems had the capability of purifying 384 and 768 compounds, respectively, it was found that doubling the number of purification channels did not double the throughput. As the purification throughput increased, postpurification sample handling was found to be the rate-limiting step.[30]

In this paper we report the design and implementation of an integrated HT purification process capable of purifying a mean of 10000 samples/month. Several key issues were

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addressed, including the handling of solvents and the hazardous waste generated, the need to automate each step of the process, the need to maximize the value of compound purification to the Institute, and the need to streamline the generation of plates for high-throughput screening.

Experimental Section

Two multiplexed LC/MS systems running MassLynx 4 were used in the course of this work, one for sample analysis and another, a Purification Factory, for mass-directed fraction collection.

Analytical LC/MS Instrumentation. The parallel four-channel analytical LC/MS system (MUX-ZQ) consisted of a 2525 binary gradient module (BGM) (Waters Corp., Milford, MA), a four-channel CTC autosampler (Leap Technologies, Carrboro, NC), a 2488 multichannel UV detector (Waters Corp.), four Sedex 75C evaporative light-scattering detectors (Richard Scientific, Novato, CA), and a four-channel MUX-ZQ (Waters Corp.). Eluents A and B were 0.05% (v/v) trifluoroacetic acid in water and 0.035% trifluoroacetic acid in acetonitrile, respectively. The BGM provided a flow rate of 5.0 mL/min that was split according to relative back-pressures of the four 50 × 2.0 mm i.d., 5 μm Ultro C18Q columns (Peeke Scientific, Redwood City, CA). After an initial 0.5 min hold at 5% B, a linear gradient of 5% B to 95% B in 3.0 min was used to elute the injected compounds from the columns. This was followed by a 0.7 min wash and a 0.8 min reequilibration time. The variation in retention times for the same compound injected on each of the four columns was within 5% of the average.

Analytical samples were created using either a Hydra96 pipettor (Matrix Technologies Corp., Hudson, NH) or a Tecan Genesis liquid handler (Tecan Group Ltd., Männedorf, Switzerland) to dilute a 0.005 mL aliquot from each sample submitted for purification to 0.10 mL with DMSO. Up to 0.01 mL of each diluted sample was injected. The LC/MS data for each sample were analyzed using OpenLynx, and the resulting report file was transformed and uploaded to an Oracle database, as described previously.[33]

Preparative LC/MS Instrumentation. Although most of the work described in this paper used one configuration for the Purification Factory, multiple configurations were examined.[34] Since the system was specified to operate from 6 to 20 mL/min per channel (24−80 mL/min total flow rate) to meet the projected needs of purifying up to 50 mg of compound/injection, the Purification Factory was located in its own solvent zone. The configuration used for most of the purification work, shown in Figure 1, consisted of a 2525 BGM, a 4X Sepmatix flow controller (Richard Scientific), a four-channel CTC autosampler, four custom splitters, a 2488 multichannel UV detector, a 515 pump (Waters Corp.) to provide makeup solvent to the ZQ inlet, a four-channel MUX-ZQ, four WFCII fraction collectors (Waters Corp.), and a custom solvent handling/waste full detection and pump shutdown system[35] (GNF, San Diego, CA). A dual-processor Xenon PC running a β version of MassLynx 4 (Build 007) was used for mass-directed fraction collection. The flow rate

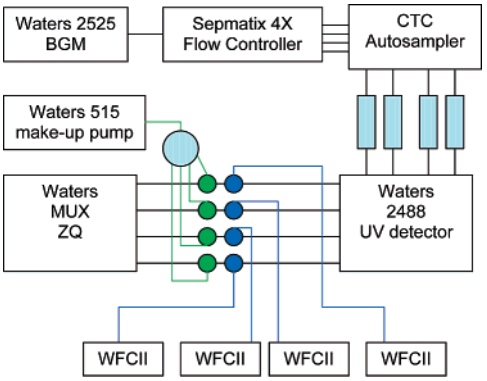

Figure 1. Purification Factory configuration used in this report.

from the 2525 BGM was 24.0 mL/min, and the Sepmatix 4X flow controller reliably split the flow among the four columns to 6.0 ± 0.1 mL/min. Since there were some pressure fluctuations observed during the injection and sample loading events, the flow controller would occasionally shut down because of its inability to control the relative flow rates to within 1.5% of the set point. This channel shutdown feature was deactivated. Typically, flow rates were within 1% of each other except during the first 30 s after the injection valves were switched to inject and the samples were being loaded onto the columns. During this time, the flow rates may fluctuate by up to 5%. Previous work[31] has shown that relative flow rate fluctuations of 5−10% have a small impact on sample recoveries.

The standard HPLC method and columns used for purification were selected on the basis of the need to purify up to 5 mg of each compound/injection. For purification of these quantities, a generic HPLC method used was 24 mL/min total flow (6 mL/min per column), with a 10−90% B gradient in 4.7 min. Eluents A and B were 0.05% trifluoroacetic acid in water and 0.035% trifluoroacetic acid in acetonitrile, respectively. Each of the samples was dissolved in 0.2−0.5 mL of DMSO and injected. With the MUX-ZQ scanning from *m/z* 200 to *m/z* 800 in 0.2 s and an interscan delay time of 0.05 s, each channel of the MUX was scanned each second. With this early version of the Purification Factory software, it was found that a peak width of >4 s was required for reliable fraction collection triggering and for acceptable sample recoveries of at least 85% of a known quantity of standard injected. The 6 mL/min flow rate was selected on the basis of limiting solvent consumption and ensuring a peak width in excess of 4 s on the 50 × 10 mm i.d., 5 μm Ultro C18 columns (Peeke Scientific). Using a procedure described previously,[30] it was found that at least 90% of the amount of Fmoc-Thr(tBu)-OH injected on each of the four channels was collected.

Custom Solvent Switching and Waste Detection System.[35] The custom solvent switching and waste detection system was designed to switch between source solvents when a source bottle runs dry and to shut down all HPLC pumps and LC/MS systems feeding a common waste container when the container is full. The unit was designed and constructed to enhance operator safety and to permit the systems to run at much higher flow rates per channel (up to 20 mL/min per channel) and still operate uninterrupted for 24 h.

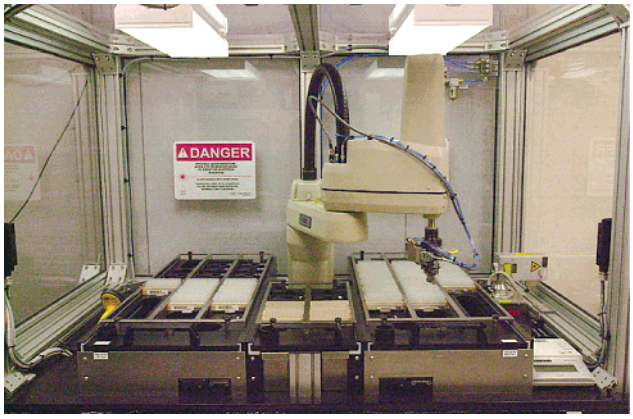

Figure 2. Custom GNF tube labeling, sorting, and weighing robot.

Labeler, Sorter, and Weigher (Figure 2). The tube sorter, shown in Figure 2, is a high-speed automated machine for labeling, weighing, and reformatting polypropylene tubes in tube racks. The nonsterile, 1.4 mL polypropylene tubes used are commercially available in 96-well latch racks (Matrix Screenmates, part number 4247, Matrix Technologies Corp.). The Matrix tube racks used in this work accommodate 96 tubes each (8 rows × 12 columns). Tube racks uniquely identified with barcode labels are manually loaded onto three trays on the deck of the instrument under the work envelope of the robot. Two long trays, left and right, accommodate 21 tube racks each. The shorter, center tray accommodates eight tube racks. A multiaxis Adept Cobra 600 robot (Servo Systems Co., Montville, NJ) with custom gripper tooling handles all the automated transfers of the tubes from the tube racks to the laser nest, from the laser nest to the balance nest, and then back to the tube racks. Before any mass measurements are made, the tubes are labeled with an ID (plate number and well location, e.g., TS123456A01) unique to the batch using a class IV Domino DSL1 sealed CO_2-type laser and returned to their original rack location. To preserve the integrity of the tube structure, the etching has been carefully calibrated to etch only the surface of the outer wall of the tube. The etching is intended to be used as an identifier of the tubes history to be read visually by an operator. This allows recovery of correct tube locations if the tubes are accidentally removed from their locations without tracking (e.g., a dropped plate). Currently, the etching is not read by any automated means. After all etching for the batch is complete, each tube is then transferred to the balance, the mass of the tube is measured, and then it is returned to the source racks. Since the tubes are composed of polypropylene, this precision of ±0.00005 g is below that achievable for the SAG285 balance[36] (Mettler-Toledo, Columbus, OH), but well within a usable range. Plastics are inherently difficult to measure with great precision due to their ability to build and hold a static charge, which influences the measurement. To reduce the effect of static charges on the tubes, two different antistatic devices are used together on the balance to achieve the best results on mass measurements. Around the top of the weigh cell tube nest on the balance is a Mettler-Toledo U-Ionizer, which ionizes air particles to reduce the static charge of material that passes through its field. In addition, two Staticmaster ionizing units (part number 2U50, available from NRD, LLC, Grand Island, NY) are placed 2 in. apart on opposite sides of the weigh cell tube nest facing each other. These units emit α particles from polonium-210 that ionize the air and help dissipate static charges. The combination of these devices has increased the precision achieved in weighing the plastic tubes used in this work. Tare masses of the empty, labeled tubes are stored in the working database. After compound solutions are transferred into these labeled, tared tubes, the solutions are dried to remove the transfer solvents in a Genevac HT-12 evaporator. Tubes with dried compound are then placed on the tube sorter, and the mass of the tube plus compound is measured to determine the net mass of compound present in the tube. The compound contained in each tube, its mass, and its purity are stored in a database. This gathering of all the data for all the tubes in a batch allows for sorting of the tubes to new destination racks based on sort designation.

Sort criteria can be flat or hierarchical on the basis of 1−10 data fields in the database. New locations are determined by sorting the stored data for each tube of the batch to achieve the desired order (e.g., number of moles of each compound, high to low) and using the sorted data to assign new locations in output plates. This enables grouping of samples (e.g., same building block, purity level, or amount), while also removing the tubes with no usable compounds. By weighing the compounds, calculating the number of micromoles of material per tube, and sorting on the basis of number of micromoles into the destination racks, the need for differential dissolution of each compound is reduced because the entire rack can be dissolved to approximately the same concentration by using the mean of the micromole quantities across the plate. Batch dissolution to a specified concentration can be done by adding the same amount of solvent to all wells of the plate if the range of the number of compound micromoles is narrow enough. If not, differential solvent handling is required to bring all wells to a set concentration. The compounds or solutions in these labeled tubes then can be readily processed by the Compound Management Group using its standard plate-replicating protocols.

Laboratory Information Management System (LIMS). A custom-built LIMS provides a suite of tools that automates data archive, robot automation support, data tracking, data query, data visualization, and report generation. The current system uses standard three-tier architecture: an Oracle database server, CGI scripts driven by an Apache Web server, and Web browsers on the client desktops. Compared to other high-throughput data sources, LC/MS data have a higher degree of complexity; therefore, it is not efficient to decompose the data into a typical relational database schema. The report files generated by OpenLynx were converted into compact XML objects (about 40 kB/sample) and stored as Oracle BLOBs. The XML technology provides a standard description of LC/MS results that is machine-independent, and reduces the complexity of the database schema, thereby improving the performance. Perl is the main programming language used to provide fully Web based interfaces for various data management and query tasks. Such Web implementations enable easy integration with other GNF

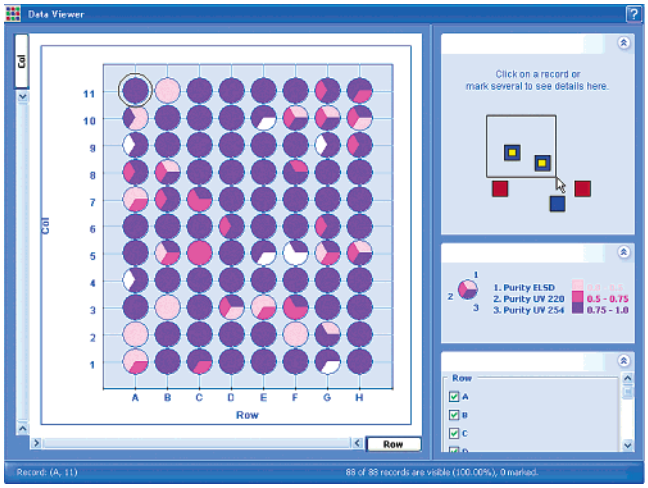

Figure 3. Plate viewer (left) and sample viewer (right) tools for interactive data visualization.

informatics systems via URL hyperlinks. Reports for selected plates and samples can be generated dynamically and exported to Excel for further analyses.

Data visualization is essential for quality control during a high-throughput process. Two platform-independent Java applets were developed for this purpose: a plate viewer and a sample viewer (Figure 3). The plate viewer can display purity information of any plate format. The relative purity of each sample well is represented by a color-coded pie chart, which contains up to three different purity criteria. Samples can be filtered on the basis of their locations, molecular weights, purities, etc. Different properties can be chosen as the plotting axes to inspect any potential correlations. Every sample of interest in the plate viewer is hyperlinked to its chromatogram data. The sample viewer provides an interactive visualization interface to the following sample information: the chemical structure, chromatograms, and identified peaks and their corresponding mass spectra. Data objects can be highlighted and zoomed; data and images in these applets can be copied and pasted into standard windows applications. These applets eliminate the workload of manual generation of static images and summary reports. Since both applets only rely on XML as the input format, they are not tied to our specific experimental setup.

Discussion

Since previous work with one-, two-, and four-column purification systems has demonstrated that sample handling, and not purification, is the rate-limiting step in a large-scale high-throughput purification effort,[30] the main focus of this work was to develop a purification process that reduces the impact of sample handling on the throughput. The key requirement of any implemented process included the ability to purify and process 2500 samples/week. It was essential that new technologies be invented and implemented to complete this goal successfully. As shown in Figure 4, the only vendor-supported solutions in place at the start of this work are shown in blue text: HT analysis and HT purification that led to either an OpenLynx report or a fraction tracking file, respectively. The lack of a suitable, commercially available integrated data management system

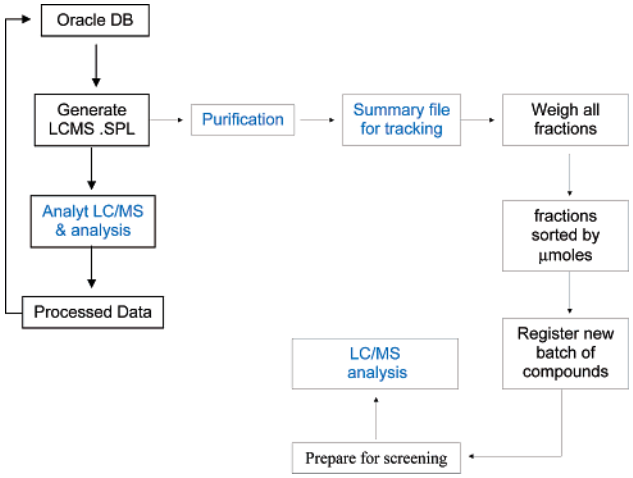

Figure 4. Vision for an integrated high-throughput purification process.

provided the opportunity not only to design an efficient workflow that helped maximize overall sample throughput but also to address other data management needs at the Institute. The assumption that purified fractions with a usable amount of material meet the purity requirements is supported by a past report that found >95% of compounds meeting the minimum mass requirement also met the >85% purity requirement.[30]

Automated Generation of LC/MS Sample Lists. All plates generated by the Compound Management Group or by individual scientists are registered in a centralized Oracle database, so that all samples that are received for LC/MS analysis or for high-throughput purification can be treated in the same manner. The process for plate submission has been standardized as the following: scan one or more of the barcoded plates and their autosampler rack locations into the analytical database, download the sample list, upload the list to MassLynx, and run the sample queue. The list generator also calculates the monoisotopic molecular masses to facilitate the automated processing associated with quality control. The resulting data are used to select the compounds for purification. The data are processed using OpenLynx, and all the chromatographic and mass spectrometric data in the report file are uploaded in the XML format to the analytical database as described above. Although the sample list generated already contains an association between the corporate ID and the sample name, the OpenLynx report is uploaded with a separate spreadsheet that maps the corporate ID to the well ID. This additional link provides the flexibility so that in the event structures are modified, the data can be reprocessed on the basis of these changes without requiring reanalysis of the samples. Using the well ID or the corporate ID, the analytical database is able to retrieve compound structural information and to publish the analytical data to other in-house lead discovery informatics systems via Web hyperlinks.

Analytical LC/MS. The analytical LC/MS data collected prior to purification were used to determine which samples should be purified and to determine the appropriate threshold for triggering fraction collection.[37,38] Samples with a purity of at least 80%, as calculated by averaging the relative purities of UV 220 nm and UV 254 nm, would be considered

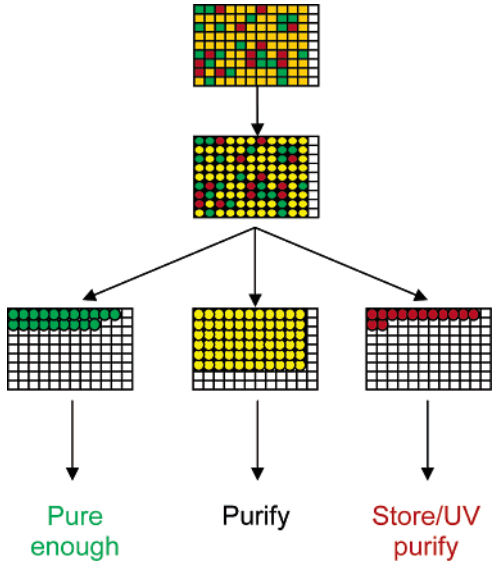

Figure 5. Sorting routine based on relative purity prior to purification.

"pure enough",[39] and after sorting, no purification would be done. Samples with a purity of less than 20% would not be purified by mass-directed purification, and after sorting, they would be set aside for further investigation. Samples found to be between 20% and 80% pure would be purified after sorting. Libraries produced internally were sorted into these three categories, shown schematically in Figure 5, and processed accordingly.

The fraction collection start and stop triggers are estimated using the intensity of the most intense peak from the analytical EIC for the desired *m*/*z* in conjunction with an experimentally determined correction factor. The correction factor is determined by comparing the intensities of a set of standards injected onto the analytical column with a scaled set of the same standards injected onto the preparative column. Due to the nonlinearity of the mass spectrometric response, there are lower and upper threshold limits imposed. These empirical rules are encoded in the LIMS so that the filenames of the fraction trigger files may be downloaded within the purification sample list for direct import into MassLynx 4.

Complete LC/MS analysis of a library is useful when planning to sort prior to purification. Sorting prior to purification was found to be useful when there were a significant number of compounds in the ≥80% purity or in the <20% purity category. Five to ten percent of each library is analyzed initially to evaluate the impact of performing 100% analysis prior to purification. For example, when the initial assessment of a group of older combinatorial libraries purchased from a compound vendor indicated that nearly all of the compounds were in the range of 30−70% purity, there was little benefit to be gained from sorting the library prior to purification. Since the EIC peak intensity was found to be comparable across the subset analyzed, additional work was not warranted. The appropriate generic gradients and triggering thresholds were determined on the basis of the data acquired. The purification success rate achieved for these libraries was comparable to that reported previously.[30]

High-Throughput LC/MS Purification and Fraction Tracking and Registration of Purified Compounds. Up to 8 plates containing 88 samples each (704 total samples) were purified per 23 h day on the Purification Factory. Due to maintenance needs and the occasional lack of samples prepared and ready for purification, this throughput cannot be sustained indefinitely. In fact, the highest daily throughput sustained for a one-month period averaged 600 samples each workday, approximately 80% of the system's theoretical capacity of 768 samples/day. The WFCII fraction collector bed size was a capacity-limiting factor, as fractions were collected into 48-deep-well plates or into custom racks,[40,41] and only two such collection racks could be accommodated on the WFCII. It is for this reason that the daily throughput generally remained closer to 528 samples/day.

To sustain this throughput, emphasis was placed on system reliability over speed. Although previous reports demonstrated that the flow from one pump can be reliably split among multiple preparative HPLC columns using relative column back-pressures to control the relative flow rate to each column,[30,31] the use of the Sepmatix 4X flow controller eliminated all flow splitting issues that may be caused by changing column back-pressures. As an initial test of the reliability of the flow controller, more than 1000 samples were run with three 50 × 10 mm i.d. columns and one 100 × 10 mm i.d. column. The flow rates and fraction collection timings remained as accurate as when the four columns were identical. Without flow control, the flow rate measured among four 50 × 10 mm i.d. column rates varied by 5−20% depending on the condition of the (guard) columns. All four (guard) columns were replaced as a set as needed. With the increase in system reliability, the daily testing of the parallel four-channel purification system decreased to collection timing checks, just as is done for a single-channel LC/MS purification system.

For this study, most of the fractions were collected into 48-deep-well plates, typically using the "1 for 1" collection method in FractionLynx and the samples tracked using a custom fraction tracking application written in-house. After evaporation, the fractions collected into the 48-deep-well plates were dissolved in 0.5 mL of 50% DMSO/methanol and transferred to tared, labeled microtubes using a Tecan Genesis liquid handler. The purified samples and their new locations were registered in the corporate database. The samples were then evaporated again using a Genevac HT-12 Series II evaporator, using conditions found to be effective in removing 0.5 mL of 50% DMSO/methanol from matrix tubes devoid of any sample. Following evaporation, the tubes were weighed to determine the net masses of the compounds. On the basis of the number of micromoles collected, the tubes are sorted to facilitate nondifferential sample dissolution in compound management. The collected fractions are assumed pure,[30,42] sorted on the basis of the number of micromoles collected, and prepared for HTS. Analysis of the purified samples is done usually by sampling one of the plates generated for HTS.

Eliminating the Postpurification Liquid Handling Steps. In the course of these purifications, parallel efforts were being made to develop and implement a custom fraction collection

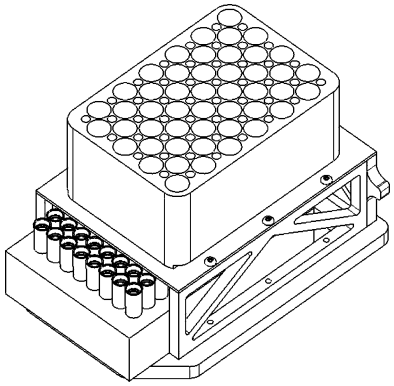

Figure 6. Custom capacity altering device to reduce postpurification sample processing.

rack that would temporarily alter the volume of the collection vessel during collection and that would separate from the final storage vessels after solvent evaporation. It is worth noting that the final tube volume of 1.2 mL is compatible with the collection of 5 mg of a compound with a molecular mass of 500 amu and with its dissolution to 10 mM in 1.0 mL of DMSO by the Compound Management Group. A schematic of this custom rack is shown in Figure 6. This rack was designed to fit into a footprint slightly larger than that of the 48-well collection plates being used, while the spacing of the openings in the top and the bottom of the adapter block were aligned with alternating labeled and tared tubes in a standard Matrix tube rack.

Thus, if samples were collected into each of the 48 wells of the custom collection block, upon solvent evaporation, the dried samples would be found, for example, in wells A2, A4, A6, A8, A10, A12, B 1, B3, etc. The body of the adapter was made of a material resistant to common HPLC solvents used and of a material light enough for use in a commercial rotary evaporator (Discovery SpeedVac Concentrator, ThermoElectron Corp). Removable modifications were made to the swings of the evaporator to support the use of this rack.

Experiments were done during the development of the rack to optimize its ability to transfer sample to the tared, labeled tubes at the bottom of the rack and to investigate what effect, if any, the adapter would have on the final weights determined. Since the largest uncertainty was expected to arise from precipitate forming inside the adapter as the acetonitrile was evaporated, experiments were done to determine the acetonitrile/water composition that would form a solution of 1.5 mg/mL Fmoc-Thr(tBu)-OH and also form precipitate during evaporation. On the basis of the results of these experiments, Fmoc-Thr(tBu)-OH was dissolved in 40% acetonitrile in water to a concentration of 1.5 mg/mL and a 4.0 mL aliquot added to each of the vessels in the custom rack. As a control, 4.0 mL of this solution was added to each well in two 48-well collection plates. Both sets of samples were evaporated to dryness, and the samples in the 48-well plates were redissolved in 0.5 mL of 50% DMSO in methanol, transferred to labeled, tared tubes, and evaporated again to dryness. For the purpose of this comparison, these samples were considered to represent 100% recovery.

From experiments done with earlier designs, it was found that Fmoc-Thr(*t*Bu)-OH would precipitate from 40% acetonitrile in water during evaporation and that the amount of compound collected in the tared tubes would be much less than that obtained from the 48-well plates. By varying the materials of the adapter body and the various apertures and angles inside the adapter, a rack was developed that was able to transmit ≥95% of the compound into the labeled, tared collection and storage tube. On the basis of these results, a shift from 48-deep-well plates to this custom rack was made.

Postpurification LC/MS Results. Figure 7 shows a summary of the relative UV 220 nm purities of samples from two less successful combinatorial libraries before and after

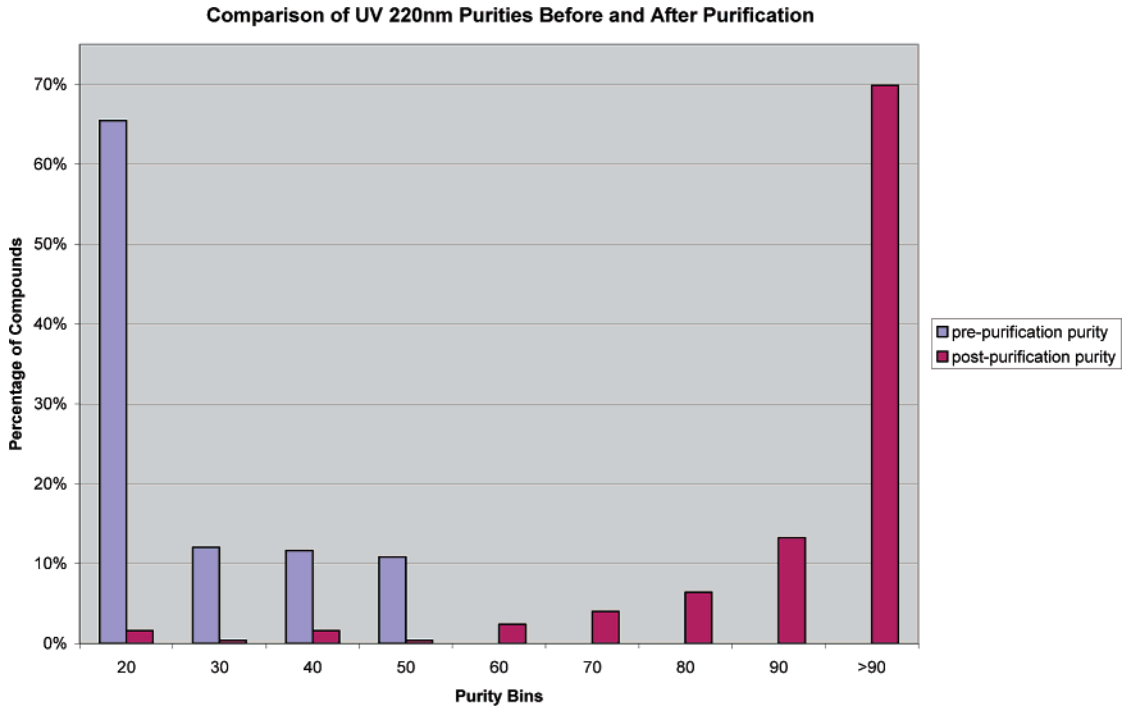

Figure 7. Relative purities of a less successful combinatorial library before and after purification.

purification. After purification, 90% of these samples were at least 80% pure by UV 220 nm. Like the single-channel LC/MS purification systems, the Purification Factory is capable of producing highly pure compounds even from low-purity mixtures.

Conclusions

A reliable, integrated, high-throughput purification process was developed that could keep pace with the throughput achievable with the Purification Factory, a four-channel LC/MUX-MS purification system. Although a peak daily throughput of 704 samples/day was achieved with this system, purification and processing of 528 samples/day was more realistic. The four-channel LC/MS purification system is capable of providing results comparable to those of four single-channel systems. The high-speed tube sorting robot provided the opportunity to maximize the impact of sample purification by limiting purification to those compounds for which it would have the greatest impact. It is also an important tool for rapidly preparing high-throughput screening plates from the purified samples because it is capable of sorting on the basis of the number of micromoles, thereby simplifying the liquid handling steps in compound management.

The purification process described herein has proven valuable to GNF, as scientists are routinely synthesizing large and complex libraries that are purified for high-throughput screening. This process has also been applied successfully to the configuration of smaller scale "pseudolibraries" consisting of cherry-picked compounds from the our proprietary compound collection selected on the basis of observed biological activity in high-throughput screening,[38] demonstrating that parallel purification and sample processing need not be limited to samples resulting from parallel chemistry.

Acknowledgment. This paper is dedicated to the memory of Matthew Rynd.

References and Notes

(1) Caldwell, J. Cellular Vehicles for Target and Drug Delivery. *Drug Discovery Technology 2003*, Boston, MA, Aug 10−15, 2003.
(2) Battersby, B. J.; Trau, M. *Trends Biotechnol.* **2002**, *4*, 167−173.
(3) Zhang, J.-H.; Chung, D. Y.; Oldenburg, K. R. *J. Comb. Chem.* **2000**, *2*, 258−265.
(4) Rishton, G. M. *DDT* **1997**, *2*, 382−384.
(5) Rishton, G. M. *DDT* **2003**, *8*, 86−96
(6) Muegge, I. *Med. Res. Rev.* **2003**, *23*, 302−321.
(7) Walters, W. P.; Murco, M. A. *Adv. Drug Delivery Rev.* **2002**, *54*, 255−271.
(8) Salmon, S. E.; Liu-Stevens, R. H.; Zhao, Y.; Lebl, M.; Krchnak, V.; Wertman, K.; Sepetov, N.; Lam, K. S. *Mol. Diversity* **1996**, *2*, 57−63.
(9) Yurek, D. A.; Branch, D. L.; Kuo, M.-S. *J. Comb. Chem.* **2002**, *4*, 138−148.
(10) Wang, T.; Zeng, L.; Cohen, J.; Kassel, D. B. *Comb. Chem. High Throughput Screening* **1999**, *2*, 327−334.
(11) Fang, L.; Cournoyer, J.; Demee, M.; Zhao, J.; Tokushige, D.; Yan, B. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 1440−1447.
(12) Cremin, P. A.; Zeng, L. *Anal. Chem.* **2002**, *74*, 5492−5500.
(13) Nikolaev, V.; Issakova, O.; Wade, S.; Sepetov, N. Automatic LC/MS Analysis of Combinatorial Libraries−A Rational Approach. *Proceedings of the 45th ASMS Conference on Mass Spectrometry and Allied Topics*, Palm Springs, CA, June 1−5, 1997; ASMS: Santa Fe, NM, 1997.
(14) Dolle, R. E.; Guo, J.; O'Brien, L.; Jin, Y.; Piznik, M.; Bowman, K. J.; Li, W.; Egan, W. J.; Cavallaro, C. L.; Roughton, A. L.; Zhao, Q.; Reader, J. C.; Orlowski, M.; Jabob-Samuel, B.; Carroll, C. D. *J. Comb. Chem.* **2000**, *2*, 716 − 731.
(15) Kassel, D. B. *Chem. Rev*. **2001**, *101*, 255−267.
(16) Lorthoir, O.; Carr, R. A. E.; Congreve, M. S.; Geysen, M. H.; Kay, C.; Marshall, P.; McKeown, S. C.; Parr, N. J.; Scicinski, J. J.; Watson, S. P. *Anal. Chem*. **2001**, *73*, 963−970.
(17) Peng, S. X.; Henson, C.; Strojnowski, M. J.; Golebiowski, A.; Klopfenstein, S. R. *Anal. Chem*. **2000**, *72*, 261−266.
(18) Breitenbucher, J. G.; Arienti, K. L.; McClure, K. J. *J. Comb. Chem.* **2001**, *3*, 528−533.
(19) Zhang, W.; Luo, Z.; Chen, C. H.-T.; Curran, D. P. *J. Am. Chem. Soc*. **2002**, *124*, 10443−10450.
(20) Pirrung, M. C.; Tumey, L. N.; McClerren, Raetz, C. R. H. *J. Am. Chem. Soc*. **2003**, *125*, 1575−1586
(21) Mukjerjee, S.; Poon, K. W. C.; Flynn, D. L.; Hanson, P. R. *Tetrahedron Lett*. **2003**, *44*, 7187−7190.
(22) Deal, M. J.; Hawes, M. C. Separation of a two phase mixture by freezing and application to combinatorial libraries. PCT Int. Appl., 1998; 20 pp. CODEN: PIXXD2 WO 9851393 A1 19981119 CAN 130:12147 AN 1998:761819.
(23) Boger, D. L.; Goldberg, J.; Andersson, C.-M. *J. Org. Chem*. **1999**, *64*, 2422−2427.
(24) Weller, H. N.; Young, M. G.; Michalczyk, S. J.; Reitnauer, G. H.; Cooley, R. S.; Rahn, P. C.; Loyd, D. J.; Fiore, D. I.; Fischman, S. J. *Mol. Diversity* **1997**, *3*, 61−70.
(25) Wang, T.; Barber, M.; Hardt, I.; Kassel, D. B. *Rapid Commun. Mass Spectrom*. **2001**, *15*, 2067−2075.
(26) Edwards, C.; Hunter, D. J. *J. Comb. Chem.* **2003**, *5*, 61−66.
(27) Yan, B.; Collins, N.; Wheatley, J.; Irving, M.; Leopold, K.; Chan, C.; Shornikov, A.; Fang, L.; Lee, A.; Stock, M.; Zhou, J. *J. Comb. Chem.* **2004**, *6*, 255−261.
(28) Zeng, L.; Wang, X.; Wang, T.; Kassel, D. B. *Comb. Chem. High Throughput Screening* **1998**, *1*, 101−111.
(29) Zeng, L.; Kassel, D. B. *Anal. Chem*. **1998**, *70*, 4380−4388.
(30) Isbell, J.; Xu, R.; Cai, Z.; Kassel, D. *J. Comb. Chem*. **2002**, *4*, 600−611.
(31) Xu, R.; Wang, T.; Isbell, J.; Cai, Z.; Sykes, C.; Brailsford, A.; Kassel, D. *Anal. Chem.* **2002**, *74*, 3055−3062.
(32) Kassel, D. B.; Wang, T.; Zeng, L. Parallel fluid electrospray mass spectrometer. PCT Int. Appl., 1999; 49 pp. CODEN: PIXXD2 WO 9965058 A2 19991216 CAN 132:30067 AN 1999:796069.
(33) Petrov, D.; Jiang, S.; Santrosyan, A.; Asatryan, H.; Chen, K.; Benner, C.; Downs, R.; Isbell, J.; Zhou, Y. Developing Analysis and Visualization Tools for Lead Discovery. Presented at the 11th International Conference on Intelligent Systems for Molecular Biology, Brisbane, Australia, June 29 to July 3, 2003.
(34) Inlet pump configurations examined included four 600 pumps (Waters Corp.), four 1525 HPLC pumps (Waters Corp.), and a 2525 BGM with a Sepmatix 4X flow controller. Fraction collectors examined with the Purification Factory included four WFCII fraction collectors (Waters Corp.), four 204 fraction collectors (Gilson), and a 4X Sepmatix fraction collector. All configurations functioned appropriately.
(35) Micklash, K. J., II; Isbell, J. (Irm, Llc, Bermuda). Fluid handling methods and systems. PCT Int. Appl., 2003. Application: WO 2003-US2379 20030124. Priority: US 2002-351821. AN 2003:605227 This system automatically switches between the source solvent containers when the

mass of one of the source containers and its solvent falls below a set amount. When the waste-full sensor detects the fluid level at a predefined level, it sends a contact closure out to stop the pump flows feeding into the possible variation in solvent densities, the waste detection system functions independently of mass and functions solely on the occupied volume of the waste container. Several of these units have functioned reliably with several known HPLC solvents and other hazardous liquids.

(36) The SAG285 specifications can be found at http://www.mt.com/mt/product_detail/product.jsp?m=t&key=A3MDg4NjM1_D.

(37) Lefebvre, P. M.; Brailsford, A.; Brindle, D.; North, C.; Cleary, R.; Potts W. B., III; Smith, B. W. Compound Purification Workflow Management and Optimization. Presented at Pittcon 2003, Orlando, FL, March 9−14, 2003.

(38) Sasher, K.; Guintu, C.; Jiang, S.; Chen, K.; Calvin, P.; Zhou, Y.; Isbell, J. J. We Don't Purify It Until You Order It: 'Just in Time' Purification in Support of Lead Discovery Biology. *Proceedings of the 54th ASMS Conference on Mass Spectrometry and Allied Topics*; ASMS: Santa Fe, NM, 2004.

(39) Yan, B.; Fang, L.; Irving, M.; Zhang, S.; Boldi, A. M.; Woolard, F.; Johnson, C. R.; Kshirsagar, T.; Figliozzi, G. M.; Krueger, C. A.; Collins, N. *J. Comb. Chem.* **2003**, *5*, 547−559.

(40) Isbell, J. J. Design and Implementation of Technologies to Purify 10,000 Samples/Month. Presented at the 6th Annual Symposium on Chemical and Pharmaceutical Structure Analysis, Princeton, NJ, Sept 21−24, 2003.

(41) Backes, B. J.; Chang, J.; Isbell, J.; Mainquist, J. K.; Shaw, C. M. Capacity altering device, holder and methods of sample processing. PCT Int. Appl., 2004; 74 pp.

(42) This assumption is not optimal and led to the development of a novel technique for postpurification sample processing that addresses this shortcoming. The paper detailing this technique is in progress and will be published elsewhere.

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