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High-Throughput Purification Platform in Support of Drug Discovery

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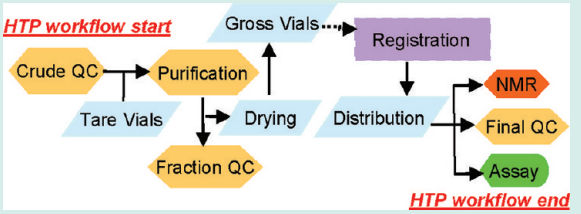
ABSTRACT: The application of parallel synthesis is an efficient approach to explore the chemical space and to rapidly develop meaningful structure activity relationship (SAR) data for drug discovery programs. However, the effectiveness of the parallel synthesis requires a high throughput purification workflow that can process a large number of crude samples within a meaningful time frame. This paper describes a high throughput purification platform that has been adopted at Merck's Rahway research site. The platform includes the evaluation of crude samples, mass-directed HPLC purification, fraction analysis, compound registration, final compound purity assessment and assay distribution. Assisting with the sample tracking and the data management is the internally designed laboratory information management system, Light Automation Framework (LAF). Using this process and the tools described herein, the group has successfully achieved purities of 95% or greater for 90% of samples.



KEYWORDS: *high-throughput purification, HPLC, mass-directed, LC/MS, parallel synthesis, instrumentation, preparative chromatography*

INTRODUCTION

The numerous challenges and prevailing headwinds facing the pharmaceutical industry are well-known and have been abundantly documented in public discourse and scientific literature over the past decade. In response to these challenges, firms, in addition to investing extensively in innovative research and technologies, are critically evaluating their operations and are adopting operating models that increase productivity and reduce discovery and development timelines. The preclinical space is much more amenable to process optimization since clinical development, which is a highly prescribed and regulated undertaking, leaves firms with few strategies that can significantly reduce approval timelines. Therefore the speed of selecting a quality preclinical candidate can profoundly impact the drug's regulatory approval, IP protection, market position and revenue curve. A common strategy that engenders operational efficiency is the creation, when appropriate, of centralized functional groups that can attain high levels of productivity through increased asset utilization and employment of subject matter experts. The high-throughput purification (HTP) group is one such entity that has been created within Merck's discovery chemistry network. The group's mandate is to expeditiously purify and isolate a large number of compounds that are destined for preclinical assessment.

Parallel synthesis has been used extensively across the industry in support of lead identification as well as lead optimization efforts.[1,2] However, it is widely recognized that purification in support of library synthesis can become the bottleneck in the process of bringing preclinical candidates forward from libraries. As a consequence, a number of approaches have been explored that have eliminated or mitigated the bottleneck of purifying large sets of compounds.[3−7] Strategies such as the use of solid-phase extraction,[8] liquid−liquid extraction,[9,10] scavenger resin,[11] or fluorous extraction[12,13] have been reported in the literature. These techniques are fast and readily automatable; however, they have their limitations. Over the years, the technique using hyphenated liquid chromatography/mass spectrometry (LC/MS)[14−27] has been widely applied in the pharmaceutical industries.

In adopting a centralized purification model it is of practical importance that the submitting chemists experience similar levels of efficiency and execution timelines as they would practice in their own laboratories. Specifically, the expectation is that compounds purified by the HTP group are delivered for inclusion in the subsequent biological assays in sufficient quantities and with a maximum purity level. In addition to meeting these criteria, the HTP group must be capable and proficient in dealing with a variety of conditions that arise in discovery sites that have a number of medicinal chemistry programs, which generally traverse the entire stages of the discovery process. In particular, challenges may be one or a combination of the following variables:[14] size of submissions as they vary widely from 1 to over 100 samples; crude scale, which can range from 5 to 100 mg, amount of the desired product as this can vary between 2% to greater than 90%; dissimilar physicochemical properties even within a library with members of purportedly similar chemical diversity, specifically, solubility, and responses of the desired product to detection by UV and MS; and the presence of impurities that can interfere with the purification via a number of mechanisms. Therefore, it is imperative to develop a flexible, robust, and high throughput

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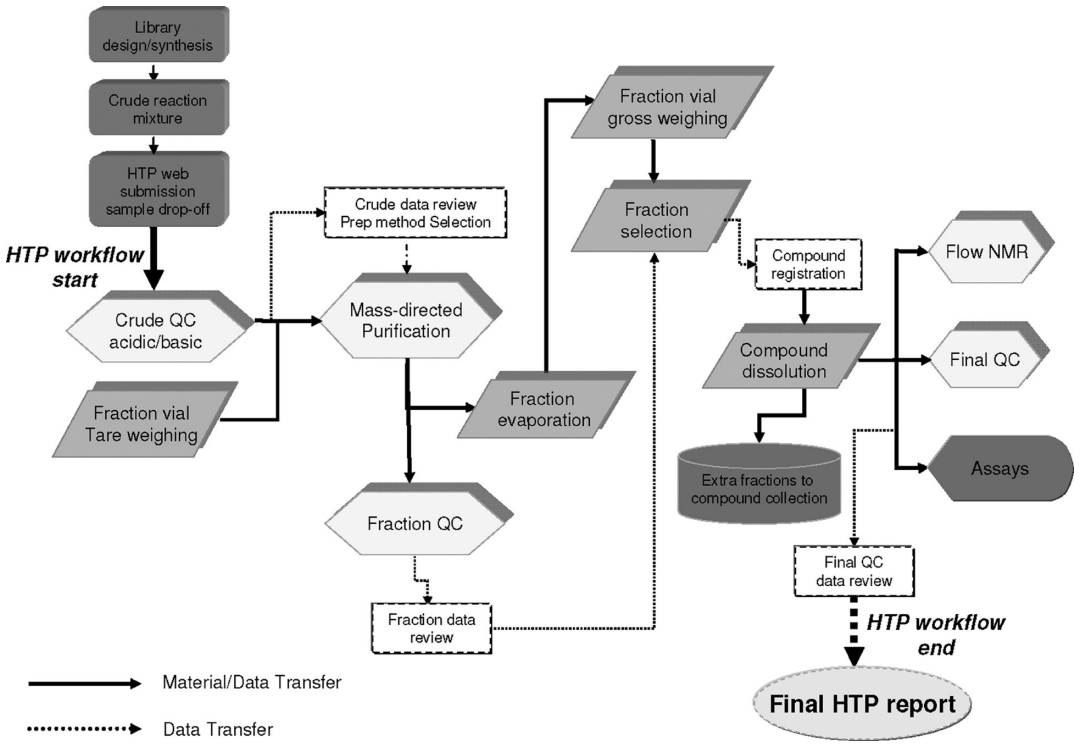

Figure 1. Schematic diagram of the high throughput purification work flow in Merck Rahway HTP group. The solid lines represent both material and data transfer. The dash lines represent data transfer. The direction of arrow represents the flow direction of the material or data.

purification process to handle all sample purification requests, regardless of the size of library, the scale of crudes, and the quality of submitted samples. In response to these challenges, our group has developed an automated HTP platform (Figure 1) that can efficiently process a large number of reaction mixtures from the crude analysis to the distribution of compounds for assays in 4.5 business days. Since the adoption of this operational model three years ago, this workflow has been used to deliver final compounds with purities in excess of 95% for 90% of the crude samples that were successfully executed.

This paper details the HTP process and the tools used to execute, manage, and track the workflow. The first part of the paper describes the workflow from crude analysis, purification, and fraction analysis to distribution. The second part of the paper discusses the involvement of LAF in the process. The final chapter summarizes the success of our platform on achieving high throughput purification in support of drug discovery in Merck.

EXPERIMENTAL PROCEDURES

Sample Submission. The first step of the purification process is the sample submission. This is accomplished by accessing the group's submission Web site shown in Figure 2. The web portal directs the submitter to input basic compound information such as project name, compound ID, molecular structure and the name of the submitter. For submission of single compounds, the information can be typed in manually. In the case of library submission, the chemist can directly upload an excel file which contains the submission information. The application will then automatically populate the required fields. After submission is performed, a notification is sent electronically to the HTP group, and concurrently an email confirmation is sent to the submitting chemist. All submission information is captured in a structural data file (SDF) in the database. Submissions are processed the same regardless of the number of compounds that are submitted. Certain guidelines are set on how samples should be prepared. In general, submitted samples need to be prefiltered, dissolved in less than 2 mL of polar solvent, such as DMSO or DMF, and delivered in high recovery vials. The crude reaction scale is preferable within the range of 10 mg to 100 mg. The purification process is initiated once the samples are physically delivered to the HTP lab.

Crude Analysis. Crude analysis is performed to evaluate whether the crude samples contain the targeted products and to determine the appropriate preparative LC conditions that should be used for purification. On average, 80% of samples of any given submission have adequate signal of the desired product making suitable for purification. The minimum passing criteria are the presence of a MS signal for the desired mass and UV threshold of 5% at 215 nm. Those that fail are returned to the chemists. Crude samples are analyzed by a Waters Acquity UPLC LC/MS system equipped with a photodiode array (PDA) detector and an evaporative light scattering (ELSD) detector. The LC separation is performed using a standard gradient of 5% to 100% of acetonitrile (all solvents are HPLC grade obtained from Fisher Scientific) in 1.4 min as the default screening method. The flow rate is set to 1 mL/min and the column temperature is set to 55 °C. Each sample is analyzed under two different pH mobile phases: at low pH modifier with 0.1% formic acid (or Trifluoroacetic acid, TFA) and at high pH modifier with 0.1% ammonium hydroxide (25% by weight as ammonium). The analysis at the low pH is conducted using a Waters HSS UPLC column (2.1 × 50 mm, 1.8 μm, C18, Waters, Milford, MA) and the analysis at the high pH is carried out using a Waters BEH UPLC column (2.1 × 50 mm, 1.7 μm, C18, Waters, Milford, MA). The crude sample is directly analyzed without diluting the samples. Upon completion of the

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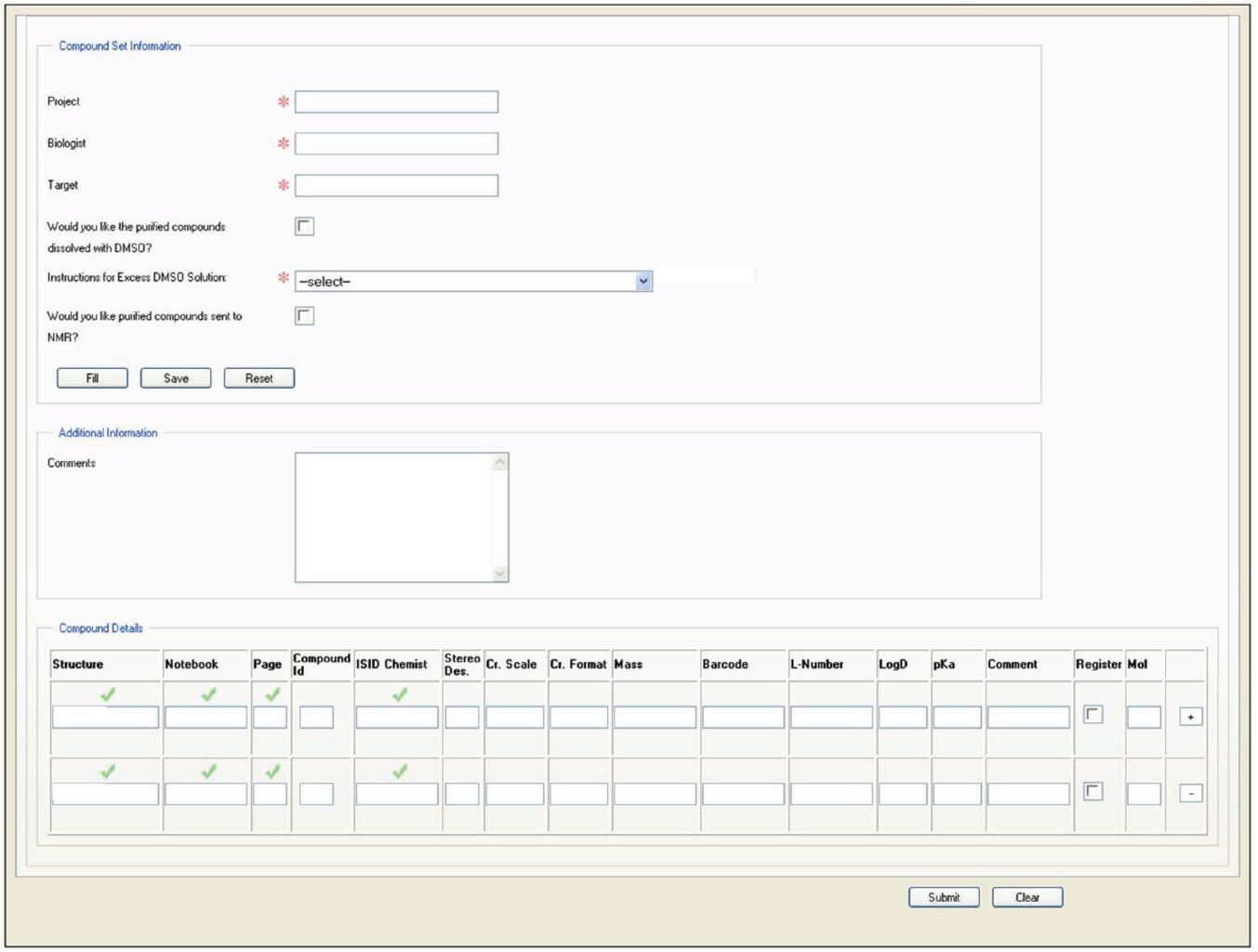

Figure 2. Merck HTP Submission Portal.

crude analysis, the PDA, LC-MS and ELSD chromatograms from each condition are compared manually through MassLynx and the one that results in the most optimal separation of the desired product from impurities is selected for performing the purification. The criteria for data evaluation under two different pH conditions include the signal intensity, retention time, peak shape of the desired product and the separation between product and impurities. An example of crude data comparison under acidic and basic conditions is illustrated in Figure 3. The desired product m/z 348 was observed at 0.68 min under the acidic condition and at 1.05 min under the basic condition. In this case the acidic condition provides a stronger product signal based on the UV response at 215 nm as well as a cleaner separation from impurities. As a result the acidic condition was selected to perform purification. A number of predetermined preparative LC methods have been developed in our lab. A preparative method that matches the crude analysis method is selected for purification.

Mass-Directed Purification. All samples are purified via a Waters mass-directed HPLC purification system. The system consists of one 2545 binary preparative pump, three 515 HPLC pumps, one 2767 sample manager with injector and collector, one system fluidics organizer, one 2998 PDA and one 3100 mass detector. The configuration and fluidic flow connections are illustrated in Figure 4.

The preparative condition is chosen by associating the retention time of the desired product in the crude chromatogram to a list of predefined preparative methods, and selecting the method with the gradient that will give the best separation. The total preparative run time is 8 min with a typical gradient window of 35% over 6 min. The modifiers used, whether formic acid, TFA, or ammonium hydroxide, are delivered via a modifier selector as defined in the method. The preparative column in the column organizer is switched accordingly based on the preparative method. The column temperature is set at 25 °C. The preparative separation at low pH is conducted on a 19 × 100 mm, 5 μm, Sunfire C18 column (Waters, Milford, MA) and the separation at the higher pH is conducted on a 19 × 100 mm, 5 μm, X-Bridge C18 column (Waters, Milford, MA). A 19 × 10 mm, 10 μm guard column (Waters, Milford, MA) packed with the same material as the stationary phase of the preparative column is used. The column flow rate is set to 50 mL/min and it consists of eluents from three sources: 1 mL/min of 8% modifier in water from an online modifier analytical 515 LC pump, 2.5 mL/min of acetonitrile from an at-column dilution 515 LC pump and 46.5 mL/min preparative flow. If a larger dimension, 30 × 100 mm, preparative column is used, the flow rate is set to 70 mL/min and is composed of 1 mL/min of modifier flow, 3.5 mL/min of at-on-column dilution flow and 65.5 mL/min of preparative flow. The selections of the

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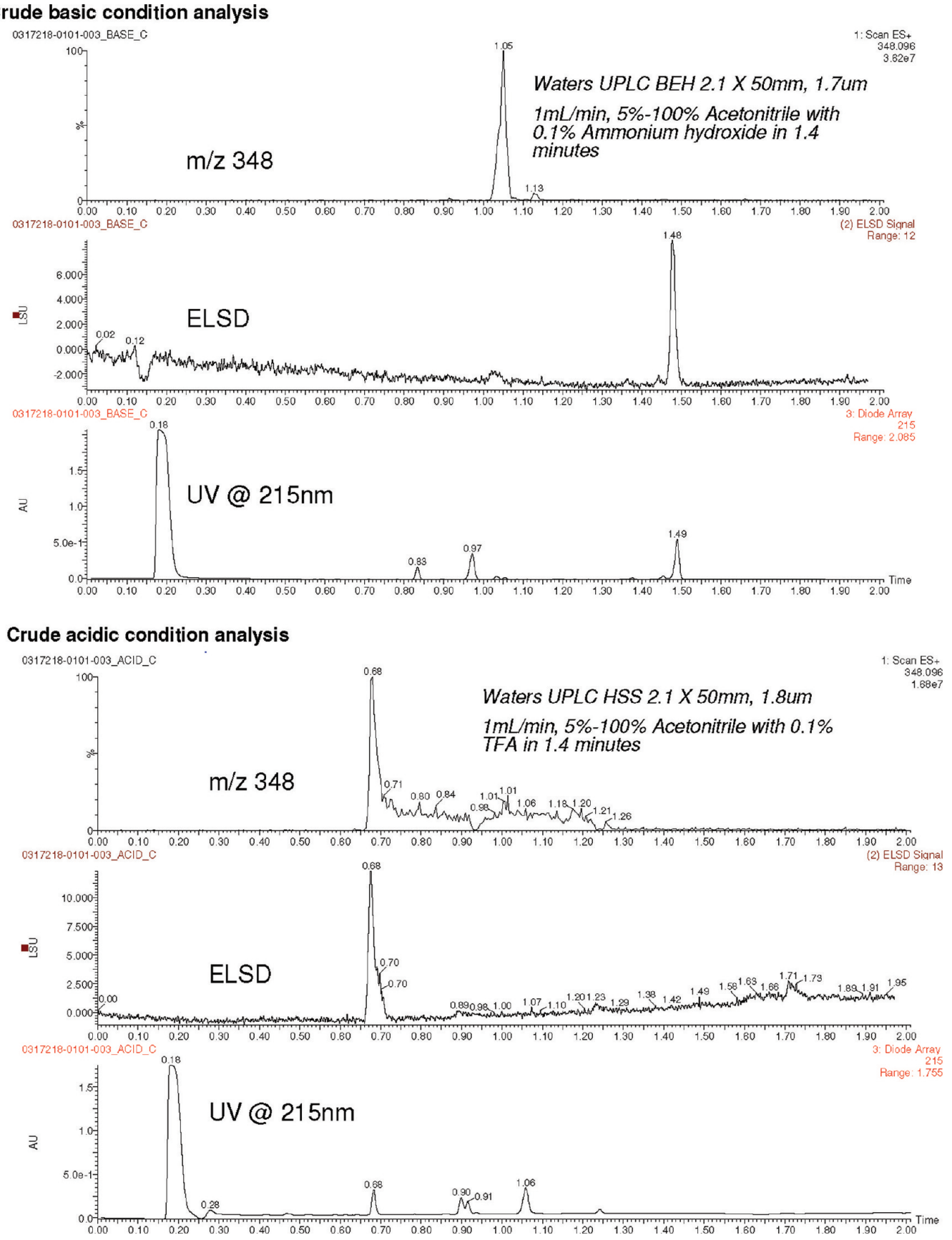

Figure 3. Example of the crude data comparison analyzed under acidic and basic conditions.

preparative flow composition, flow rate and the dimension of column are based on the crude scale, indicated in the submission.

A static splitter (30−100 mL/min, Waters, Milford, MA) with a 5000:1 ratio sends the stream to the PDA and the 3100

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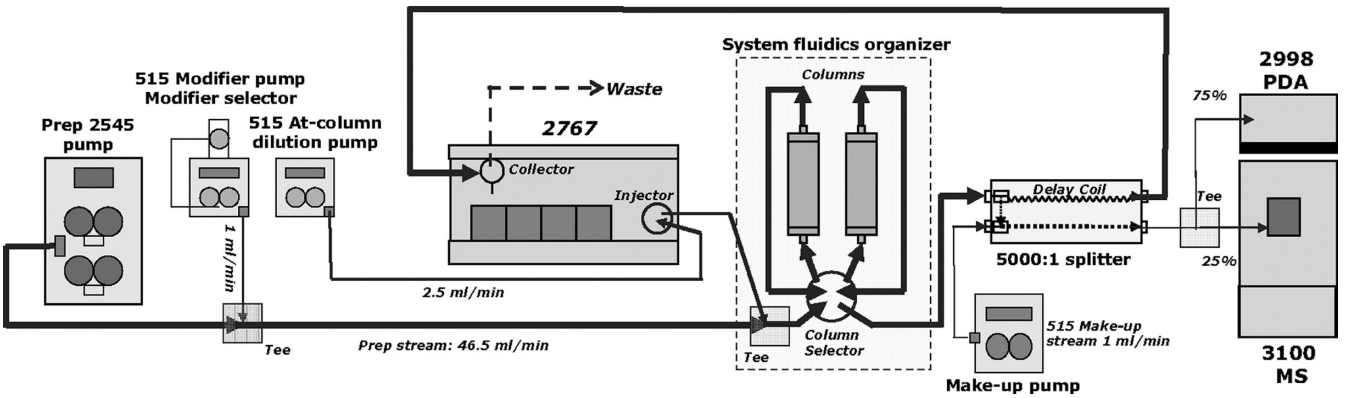

Figure 4. Fluidic configuration of the mass-directed reverse-phase HPLC purification system. The main system consists of a preparative pump, modifier pump, at-column-dilution pump, system fluidics organizer, makeup pump, PDA/MS detector, and fraction collector/injector. The main flow is indicated in heavier weight solid lines to represent the flow of preparative stream. Analytical or auxiliary flows are indicated in lighter weight lines. Flow rates are indicated next to the connection lines.

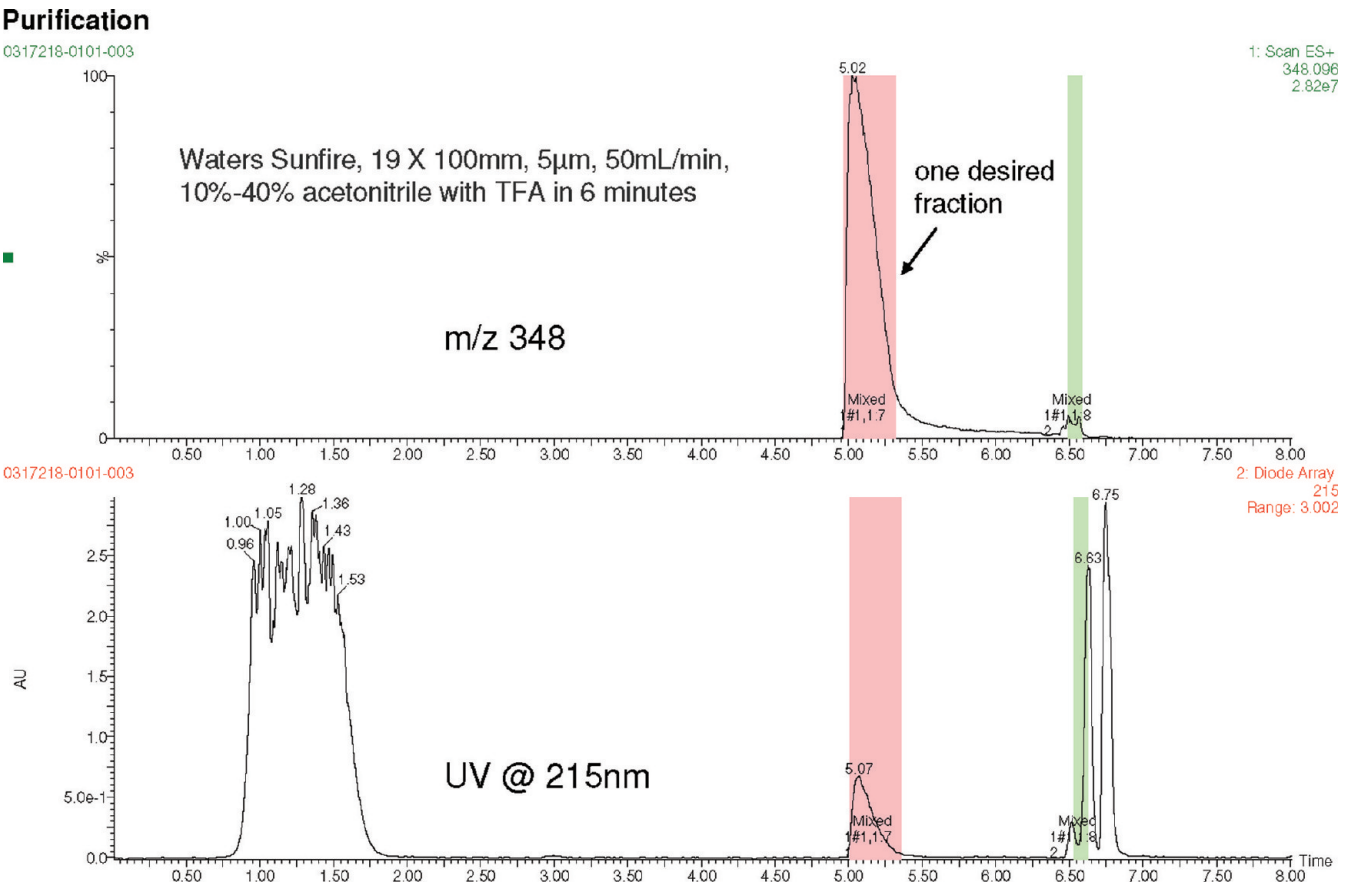

Figure 5. Example of the purification data.

mass spectrometer in parallel. A makeup 515 LC pump that delivers a constant 1 mL/min flow of methanol is connected to the splitter to dilute the flow to the PDA and mass spectrometer to avoid signal overloading. When the desired compound observed on the PDA and the MS meets the minimum collection triggering criteria, a collection is triggered. The fractions are collected in pretared barcoded 20 mL high recovery vials. The purification was performed on the aforementioned sample and the chromatograms are shown in Figure 5. The number of fractions per sample typically varies from 1 to 5 based on the preparative peak shape. Four racks of 32 vials are available for collection on the open-bed fraction collector, resulting in a capacity of 128 available vials for collection. If the number of fractions exceeds 128 in one library, the library will be divided into two sets. On average, 90% of samples are purified with the first pass and those samples that are unsuccessfully purified the first time, which typically contain closely eluting impurities to the desired product, require repurification under different conditions. The success rate of repurifications is over 95%.

Fraction QC. LC-MS analysis is performed on all collected fractions to determine the purity of the fractions. The analysis is

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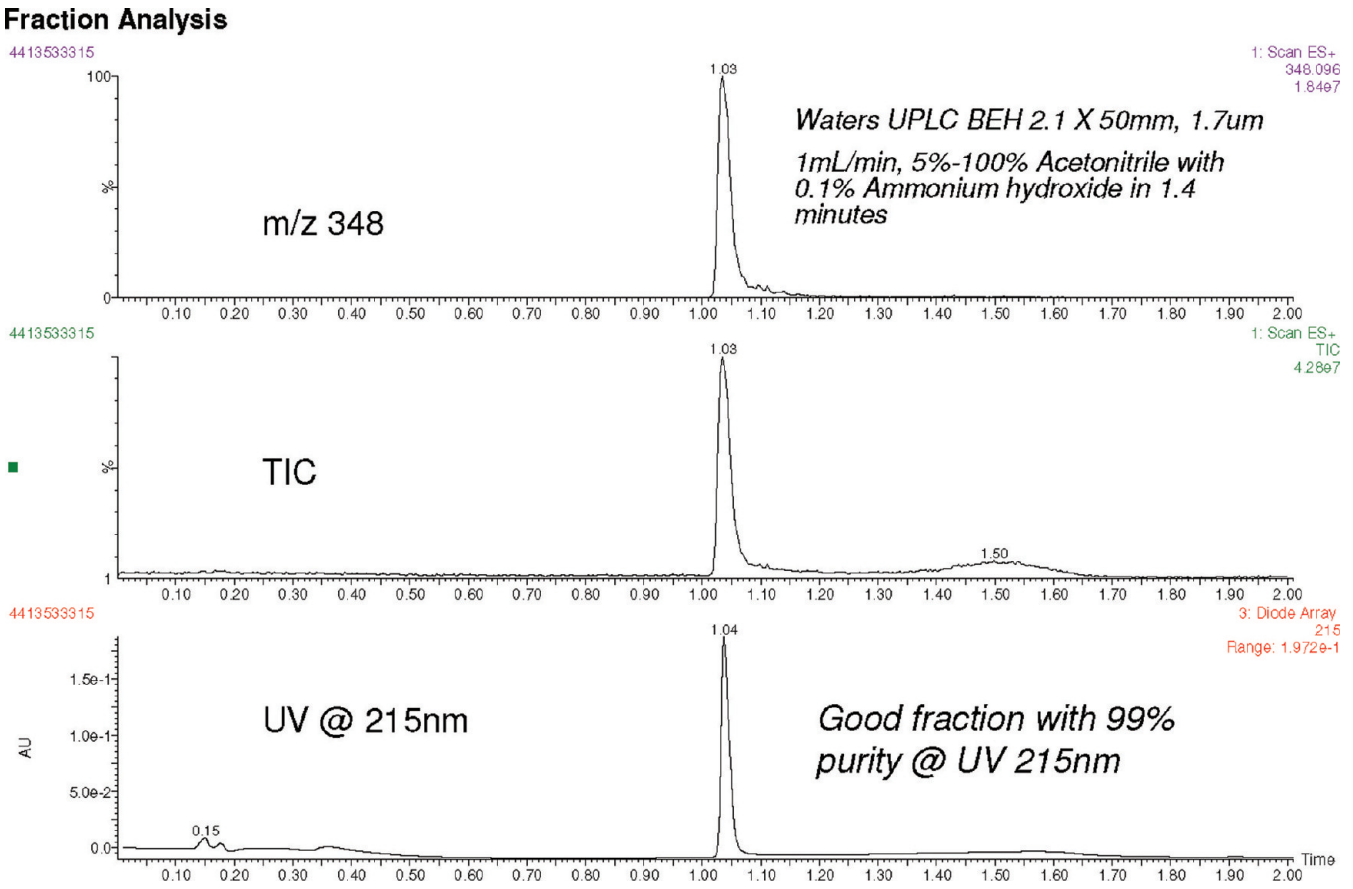

Figure 6. Example of the fraction data. The purity is 99% based on the UV response at 215 nm.

carried out under the basic condition with a gradient of 5% to 100% of acetonitrile in 1.4 min at 1 mL/min flow rate and the column temperature is set to 55 °C. This analysis is an important part of the HTP process since it not only provides the purity for each fraction, but it also acts as a gate keeper to avoid missing any fractions of interest. An aliquot of 100 uL of each fraction is transferred into a 96 well plate via a Tecan 150 (Tecan Group Ltd., Switzerland) for the fraction analysis which is performed on a Waters Acquity UPLC LC/MS. The analysis is performed overnight while the fractions are being dried in the GeneVac (GeneVac-SP Scientific, U.K.). The Tecan is configured to receive Waters fraction collection racks directly, thus, preventing the potential error of misplacing fraction vials during vial transferring. The data are reviewed manually the next morning. The cutoff for purity is set at 90% based on UV response at 215 nm. Figure 6 is an example of the fraction chromatograms of the sample that the crude and purification data are shown in Figure 3 and Figure 5. The example shows that the purity of the fraction based on UV response at 215 nm is over 99%.

Evaporation, Weighing, and Distribution. The resulting fractions are dried in 16 h on a HT24 series GeneVac by employing a programmable lyophilization method. The fraction drying method is optimized to ensure the minimum drying time while maintaining sample integrity.

Two balance automators, Bohdan Balance Automator (Mettler Toledo, Columbus, OH) and Flexiweigh station (Mettler Toledo, Columbus, OH), are used for taring the 20 mL barcoded vials as well as the resulting dried fractions. Both weighers are configured to receive the Waters fraction collection racks directly, again, to prevent the potential error of misplacing fraction vials during vial transferring.

The HTP group is also responsible for registering final compounds on behalf of submitting chemists, distributing them in 10 mM DMSO solution to the assay group, and submitting an aliquot of each compound for NMR analysis. To arrive at an accurate concentration, the resulting salt form of the compound is used by LAF to calculate the volume of DMSO. The dissolution process is handled by a Tecan 200 robot (Tecan Group Ltd., Switzerland). An ultrasonic bath (Model FS220H, Fisher Scienific) is used to facilitate sample dissolution. Solutions are transferred into 4 mL V-bottom high recovery vials for assays, into a 96 well plate for the final LC/MS QC plate and into a 96 well plate for the NMR analysis. The final QC is processed automatically by Waters OpenLynx software and the processed report (.rpt) is saved on the server for chemists to review and to import to the electronic notebook. Figure 7 is an example of the final QC performed on the fraction selected from the preparative data shown in Figure 6. The final purity of the distributed 10 mM DMSO solution is 99% based on the UV response at 215 nm.

DISCUSSION AND RESULTS

Light Automation Framework (LAF). The centralized HTP group has the skill set to isolate desired products from crude reaction mixtures. However, the crude reaction mixtures often have extremely complex separation profiles that can pose enormous analytical challenges to the HTP process and can lead to multiple fraction collections per sample. The complexity can quickly add up to a nearly unmanageable level in large

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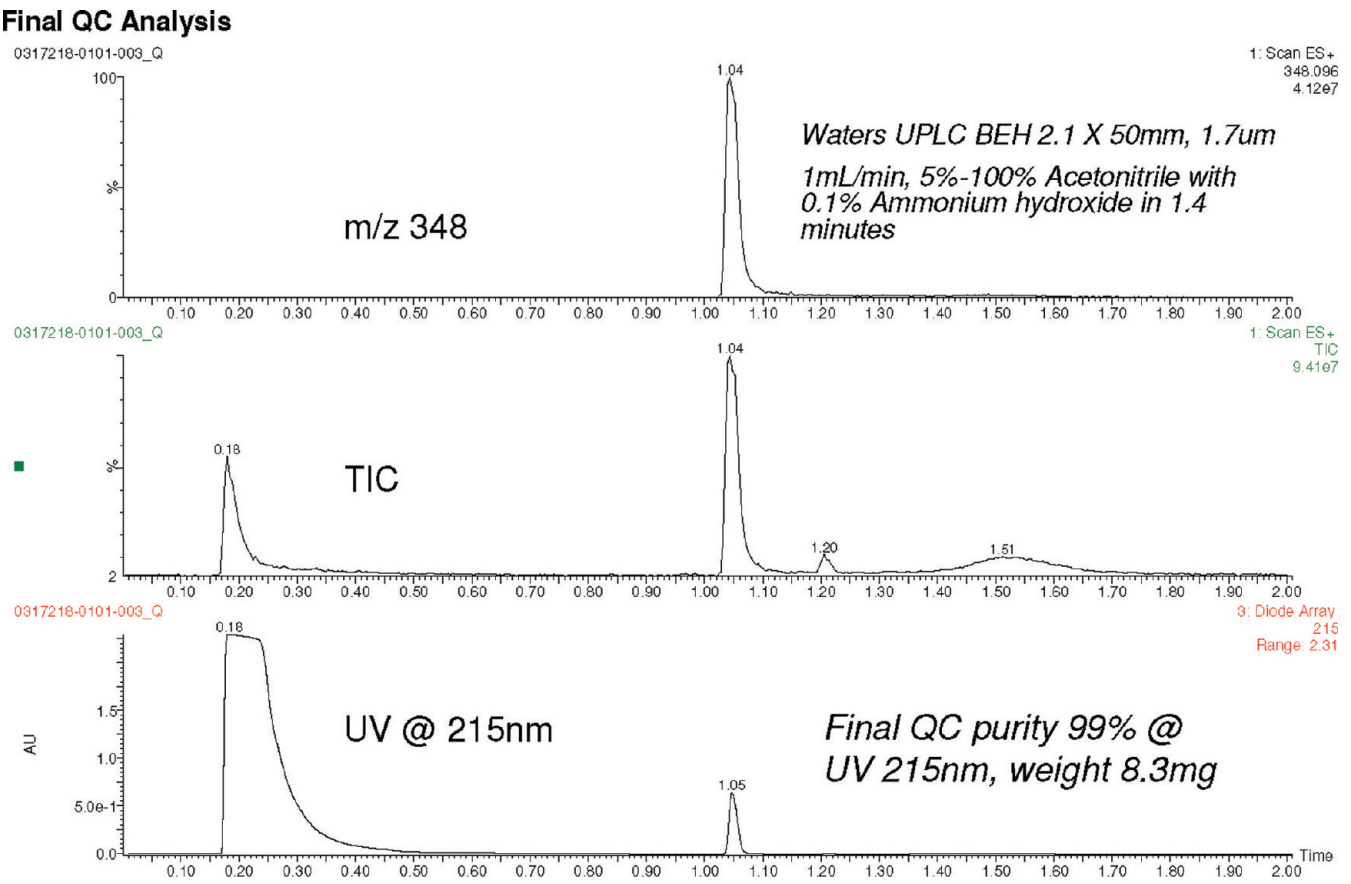

Figure 7. Example of the final QC data. The purity is 99% based on the UV response at 215 nm.

library purifications. Therefore, a tool capable of tracking the progress, identifying, and locating all the fractions is critical to achieve high throughput and high efficiency. A laboratory information management system (LIMS), light automation framework (LAF) is designed to fit this need to support HTP library purification.

LAF is Java based and is integrated with an Oracle database. As shown in Figure 8, LAF controls the information flow in every step of the HTP process: it sends project information to each instrument station and collects data back to the central server for review and decision making. LAF has been designed to process the work flow in two distinct ways: submission based work flow and function based work flow. The submission based work flow treats one submission as a whole such that one HTP individual works on the entire process of one submission from the crude analysis to the final report. The function based work flow divides one submission into several function based processes and each individual only works on a particular function of the submission. The primary advantage of the function based work flow is that its working mechanism does not distract people from one function to others hence work can be done at a much faster pace. Therefore, the function based workflow is the preferred process in our group and has been working well in production. The key to the success of the function based work flow requires a high degree of coordination and constant communications among multiple people during work transferring from one function to another, which has been accomplished successfully in our group.

LAF has several unique functions in facilitating purification process. First, LAF contains all information pertaining to all the compounds from different submissions. After LAF processing, the submission is referred to as a "set" and every set has two levels of information listed in LAF: the compound level and the set level. The information for the compound level is focused on individual compound data such as compound structure, exact mass, crude scale, crude format, specific comment, etc. The information at the set level includes program, assay, distribution information, etc. Therefore, LAF can be used as a reference database to track historic data if needed.

Second, LAF interfaces with every step of HTP work flow. All analyses are acquired by Waters MassLynx software, which is project based with each project containing three major folders: ACQUDB, DATA and SampleDB. LAF can directly create a project folder on the local instrument computer in MassLynx. The project for the analytical analysis, including the crude, fraction and final QC are generated on the local Acquity computer. This is also the case for project generation on the purification systems. Then, all the default preset methods stored in the LAF database can be automatically transferred to the ACQUDB folder in that project. For example, all of preset preparative methods are automatically transferred to the ACQUDB folder in the purification project. The appropriate preparative method can be directly selected in MassLynx from the preset methods on the local preparative computer. The SampleDB folder which is also created by LAF contains a sample list with sample name, compound exact mass, LC and MS method, injection location and injection volume, etc., automatically populated for data acquisition. The data transfer from each local instrument computer to the LAF server is performed automatically. The folder of the crude data is named

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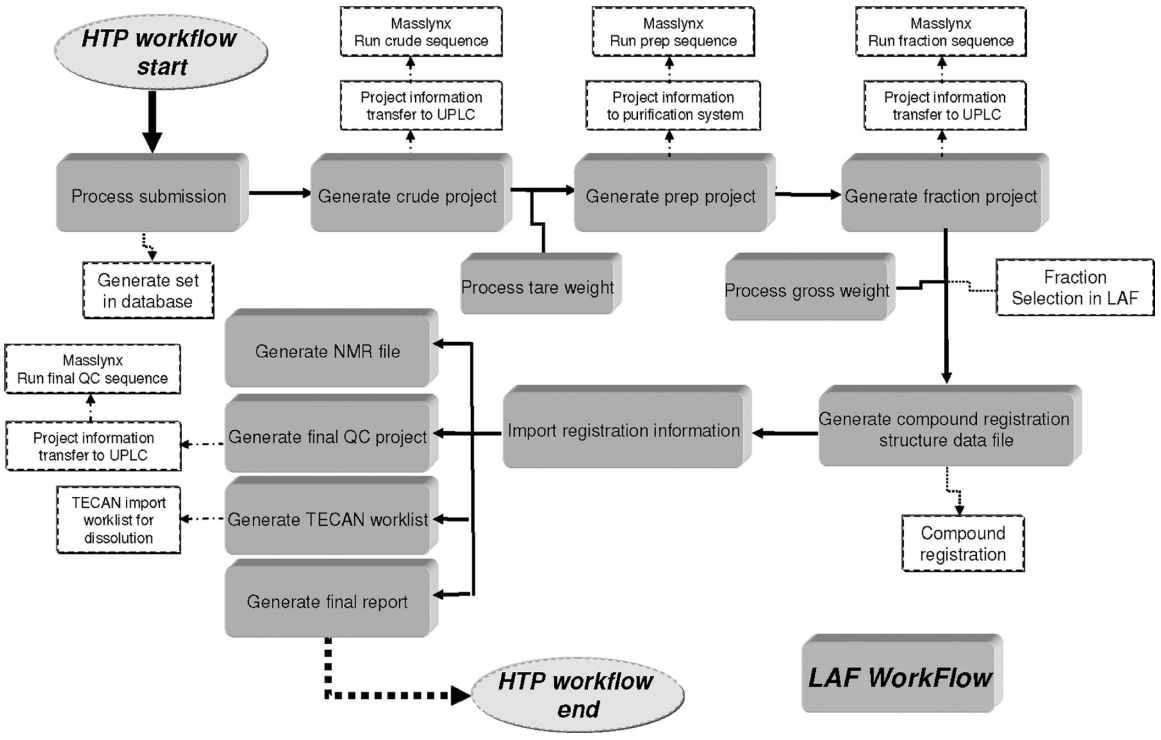

Figure 8. Illustration of the involvement of Light Automation Framework in each individual process of the HTP workflow. LAF controls the information and data flow in the whole HTP process.

"set_crude", the purification data is called "set_prep", and the fraction data is stored in the folder "set_fraction". All data generated from each process are automatically copied from the local instrument computer to the LAF server and saved under the name of the same set making convenient to locate the data on the server. Because of this network interface between LAF and the instruments, it becomes straightforward to retrieve data from every individual step of the HTP work flow.

The third level of LAF's function is its ability to extract fraction information from purification raw data (.raw) and correlate corresponding fraction vial barcodes, vial locations and vial weights. The tare weight file processed by LAF lists vial barcodes, tare weights and their predefined vial location. The fraction location in the purification data is determined by the order of vials listed in the tare file. LAF can read fraction information in raw data and associate each fraction based on its location with its vial barcode and vial weight via the tare weight file, and then export all information to a single file for easy review.

The last step in LAF is registration and distribution. LAF can generate a SDF for compound registration which can be directly imported to the Merck compound registration system for batch registration, a TECAN work list for compound dissolution which contains volume of DMSO automatically calculated by LAF, a NMR file which can be directly uploaded to the NMR instrument for data acquisition, and a distribution SDF for compound distribution. Finally, LAF gathers all information from the whole process and compiles them together in an excel report which is sent to the submitting chemist via email to conclude the HTP process.

CONCLUSION

The establishment of a central purification group has allowed medicinal chemists to explore the relevant chemical space around their lead series in a rapid and efficient manner. Furthermore, by performing the ancillary functions that were once carried out by synthetic chemists, the HTP group has freed more of their time so that they may focus on the design and synthesis of compounds of higher complexity or address issues that hamper program progression.

Since the group's inception, approximately three years ago, this high throughput purification platform has allowed for the purification of greater than 40 000 compounds, resulting in a purity of 95% or greater for the 90% of the compounds purified. With the current resources the group's capacity is 30 000 compounds per year with over 95% of the submissions processed within 4.5 business days.

To be efficient in this endeavor, the HTP follows a predefined workflow. This platform processes single compounds or library submissions using a function based work flow including crude analysis, purification, fraction analysis, compound registration/distribution, and the final purity assessment. To manage the workflow and data resulting from this process, LAF, the LIMS system was developed in-house. The software is also unique in that it is capable in dealing with multiple fractions per sample.

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