AN AUTOMATED METHOD FOR SAMPLE PREPARATION FOR GC ANALYSIS USING SOLID SCAVENGERS

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Abstract

Sample preparation plays a pivotal role in chemical analysis by significantly influencing its quality. This preparatory stage is not only labor-intensive but also a critical component of most analytical processes. The need for automating sample preparation becomes evident, primarily to boost the efficiency of chemical analyses, maintain their precision and accuracy, and, crucially, reduce the risks associated with human exposure to potentially hazardous chemicals or biological substances.

In the domain of gas chromatography, a prevalent challenge arises due to certain harmful compounds. These problematic substances, often highly polar and reactive, can detrimentally interact with the surface of the chromatographic column, leading to its degradation. Overcoming this obstacle is essential for the widespread use of gas chromatography. The solution lies in the efficient separation of these reactive compounds, for which scavengers, available in both immobilized packed column and bulk powdered forms, prove indispensable. These scavengers selectively attach to the reactive compounds, effectively removing them from the sample before it enters the gas chromatograph. This study presents the development of an automated sample preparation method designed to eliminate residual 4-methoxyphenylboronic acid from an oxidative Heck reaction mixture. In this method, powdered SiliaBound Diol resin was determined to be the scavenger of choice. Through a systematic approach, we optimized various parameters, such as the choice of solvent, the quantity of scavenger used, the concentration of methoxy phenylboronic acid, and the number of aspiration-dispensing cycles. These optimizations were geared toward achieving maximal efficiency in the removal process, with a remarkable outcome of approximately 95% extraction of boronic acid from the mixture.

Keywords: GC Sample Preparation, Automation, Scavenger

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1. Introduction

1.1. Background

In the domain of analytical chemistry, the primary objective of any analysis is to gain insights into the composition of a sample. At its core, sampling is the art of selecting a representative portion from a larger whole. Through meticulous sampling, scientists aim to decipher the intricate details of a mixture. It is not merely about knowing what compounds are present, it's about understanding the quantity of each compound. In other words, sampling is the initial step toward answering critical questions about the mixture, i.e., what compounds exist in the sample, and in what quantities?

The synergy between sampling and analytical instruments is undeniable. Analytical techniques, such as gas chromatography, liquid chromatography, and NMR spectrometry, rely heavily on the quality of the samples they receive. The importance of sampling becomes even more apparent when we consider the diversity of these instruments and their unique requirements. Each analytical method demands a tailored approach to sample preparation. For instance, gas chromatography necessitates specific considerations (such as physical state of the sample, chemical or thermal stability, sample volume, etc.) that differ from those of liquid chromatography or NMR spectrometry. The success of any analytical method hinges on the meticulous preparation of samples to suit the requirements of the instrument in use.

1.2. Gas Chromatography and the Importance of Sample Preparation

1.2.1. Gas Chromatography: A Cornerstone of Analytical Insights

Gas Chromatography (GC) is a foundational technique in analytical chemistry, offering unparalleled capabilities in the separation, identification (by comparing retention time), and quantification of volatile and semi- volatile compounds within complex sample matrices. A gas chromatograph comprises an injector, columns, an oven, detectors, a control system, and the carrier and make-up gas pneumatics (**Figure 1.1**). A syringe or gas manifold

introduces an analyte(s) into a heated headspace to ensure that it is in the gas phase. An inert carrier gas (He or H_2) (mobile phase) transports the analytes from this chamber to the columns housed in a furnace that controls the temperature. The analytes adsorb and desorb in the stationary phase as they travel along the column along with the mobile phase. The rate at which they desorb and absorb determines how long it takes for them to elute and reach the detector (**Figure 1.2**).[1], [2] This powerful method is built upon the principle of differential partitioning, where compounds distribute between a mobile gas phase and a stationary phase based on their unique physicochemical properties. Widely applied across scientific domains including environmental monitoring,[3], [4] the petroleum industry,[5] pharmaceutical analysis,[6] food quality control,[7] flavor and fragrance analysis,[8] and forensic analysis, e.g., screening for drugs of abuse in blood samples,[9] GC's success is intrinsically linked to a fundamental yet often underestimated aspect: sample preparation.

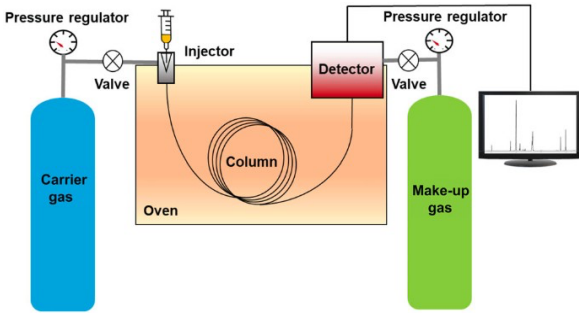


Figure 1.1 The essential features of a gas chromatograph are columns, an oven, carrier gas, and detectors. Make-up gas stabilizes the detector signal. (Diagram used in whole from reference [1])

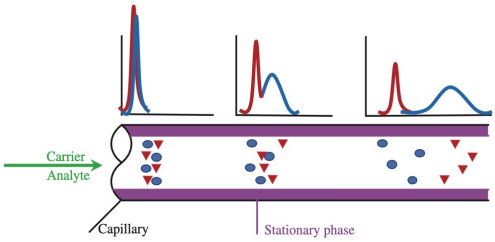


Figure 1.2 Gas chromatograms. As the carrier gas travels with the analytes along the column, the affinity that each compound has with the column determines how long it takes for it to elute. Compounds that have a higher affinity come out later and have broader and shorter peaks, where compounds with little affinity come out sooner. (Diagram used in whole from reference [2])

1.2.2. The Crucial Role of Sample Preparation

The significance of sample preparation in GC analysis cannot be overstated. It serves as the vital bridge between real-world samples [55] and the precise analysis achieved by GC. Complex samples, containing varying compounds at different concentrations, necessitate efficient preparation to ensure accurate and reliable chromatographic separation and detection. Effective sample preparation serves multiple critical objectives.[10] One of the primary goals of sample preparation before GC analysis is therefore to eliminate matrix components, which are nonvolatile and otherwise incompatible with introduction into the GC. The resulting sample fraction ideally contains the analytes of interest and can be introduced easily into the GC inlet. A task commonly associated with this goal of separating the analytes from the matrix is to improve recovery of the analytes from the matrix.

A second goal of sample preparation is to concentrate the sample -specially for trace level analysis of volatile organic compounds- prior to GC injection to achieve a desired detection limit. This aim is complicated by the fact that target analytes are often in low concentration relative to the bulk sample and therefore it is necessary to find a means of selectively concentrating the analytes while minimizing the amount of matrix (other compounds in the mixture) that is also concentrated. An example of early instrumentation to automate such a process is the purge-and- trap technology typically employed where simple headspace analysis of a liquid sample provides insufficient sensitivity.

A third significant goal of sample preparation for GC analysis is the enhancement of analyte signal intensity or quality of chromatographic analysis often accomplished through derivatization procedures. While the continual advancement of chromatographic phases and the prevalence of mass spectrometers in GC have diminished the urgency of this goal, it remains pertinent for certain compound classes. For instance, compounds like organic acids may require conversion into their esters to facilitate analysis.[11], [12]

1.3. Sample Preparation Techniques for GC Analysis

The real-world samples subjected to gas chromatographic analysis encompass a vast array of matrices and analytes. These matrices range from complex biological fluids to environmental extracts to industrial products. Meanwhile, the analytes of interest may span across volatile organic compounds (VOCs), semi-volatile compounds, and other target analytes with distinct physicochemical properties. To accommodate this diversity, a multitude of sample preparation techniques have been developed, each tailored to address specific challenges.

Techniques such as solid-phase microextraction (SPME), liquid-liquid extraction (LLE), solid-phase extraction (SPE), derivatization and headspace sampling provide researchers with a versatile toolkit for effectively preparing samples for GC analysis.[13], [14]

1.3.1. Solid-Phase Microextraction (SPME)

A powerful technique, SPME eliminates the need for extensive solvent use. It employs a fiber coated with an absorbent phase, which adsorbs analytes when exposed to the sample. Desorption takes place in the GC injector, facilitating analyte transfer for analysis. SPME is rapid, solvent-free, and particularly suitable for trace-level analysis due to its ability to enhance sensitivity.[15]

1.3.2. Liquid-Liquid Extraction (LLE)

LLE involves partitioning analytes between two immiscible liquid phases. This method effectively separates compounds with varying polarities. However, it can be time-consuming and necessitates cautious solvent handling. LLE remains a robust option for specific applications demanding distinct polarity-based separations.[16]

1.3.3. Solid-Phase Extraction (SPE)

Utilizing a solid adsorbent, SPE selectively retains analytes while washing away interfering compounds. It offers flexibility and accommodates a wide range of sample matrices. SPE is especially advantageous for concentrating analytes from large sample volumes, enhancing their detectability within the GC system.[17]

1.3.4. Derivatization

Derivatization plays a vital role in enhancing the detectability of analytes with low volatility or weak responses in GC. By chemically modifying analytes, their volatility, stability, and sensitivity are improved, leading to sharper chromatographic peaks. This technique is indispensable for compounds requiring augmented detectability.[18]

1.3.5. Headspace Gas Chromatography

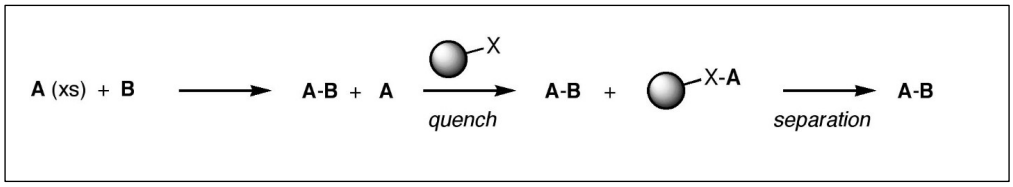
Headspace GC is the method of choice for volatile and semi-volatile compounds. It involves analyzing the vapor phase above a sealed sample vial. Particularly prevalent in industries such as food, beverages, and environmental analysis, this technique ensures accurate determination of volatile analytes.[19]

1.4. Solid-Supported Purification Techniques: The Role of Scavengers in Sample Preparation

Most chemical reactions need work-up and purification, a process through which chemists spend a significant amount of time. Techniques that simplify and expedite these operations can significantly enhance a chemist's productivity, allowing for more creative work and increased output. Solid-supported systems, acting as scavengers, quenching agents, and catch-and-release platforms, have emerged to assist chemists in these endeavors.[20]

1.4.1. Scavengers

Scavengers sometimes referred to as sequestering or quenching agents, are reactive substances that selectively quench or remove reaction by-products or excess starting materials. They can be easily removed by filtration. For example, the product of a reaction between compound B and an excess of compound A will be AB contaminated with the remaining excess of A. After reaction completion, isolation of AB from this excess reagent can be achieved by applying a suitable scavenger resin. The scavenger reacts with excess A by bonding to it in some way. A simple filtration separates the solution of AB form scavenger resin sequestered A (**Scheme 1.1**).[21] Solid- supported scavengers, particularly insoluble polymers, play a pivotal role in scavenging by- products and excess reactants from complex reaction mixtures, simplifying purification without the need for liquid-liquid extractions or non-specific column chromatography.



Scheme 1-1 Scavenger resins to assist in the removal of excess reagents.
(Diagram used in whole from reference [21])

Two common classes of scavengers include those forming ionic interactions, such as acidic and basic resins (ion-exchange resins), and those forming covalent bonds, including electrophilic and nucleophilic species.

Standard scavenging procedures often rely on complementary reactivity. For instance, electrophilic and nucleophilic species can be effectively sequestered using reciprocally functionalized solid supports, while acids and bases can be removed by forming salts with solid- supported bases or acids. Supported reagents have not only been employed to scavenge organic fragments but have also shown potential in selectively binding ions or inorganic complexes from solution.[22], [23]

1.4.2. Modified Scavenging Protocols

Modified scavenging protocols come into play when reactions involve poorly reactive starting materials, resulting in product mixtures containing challenging-to-remove reactants. In such cases, adding a highly reactive bifunctional reagent to the mixture can transform the less reactive compound into an easily trapped intermediate, which is subsequently removed by a conventional scavenger. Both electrophilic and nucleophilic reagents have been used in this manner.[20]

1.4.3. Catch-and-Release Purification Methods

Conventional scavenging method is being considered as a purification method for rapid clean-up of reaction mixtures through sequestration of unwanted compounds including by-products, excess reactants or spent reagents. In catch-and-release strategy, suitably functionalized solid supports are used to selectively capture the desired product away from impurities, filter it, and then release it in a pure form. Various mechanisms, both reversible physical and chemical adsorption, can be employed for trapping and releasing the product. This approach has been adopted in commercially available solid-phase extraction (SPE) kits, enabling efficient purification for various applications including chemical library preparation

for biological screening.

As synthetic mixtures become more complex, efficient work-up and purification becomes increasingly challenging. The methods discussed here are valuable additions to the toolkit of rapid purification techniques, which can be applied independently or in conjunction with other supported-reagent systems. Adoption of scavenging protocols extends beyond combinatorial and parallel synthesis, becoming increasingly common in diverse areas of synthetic chemistry.[20]

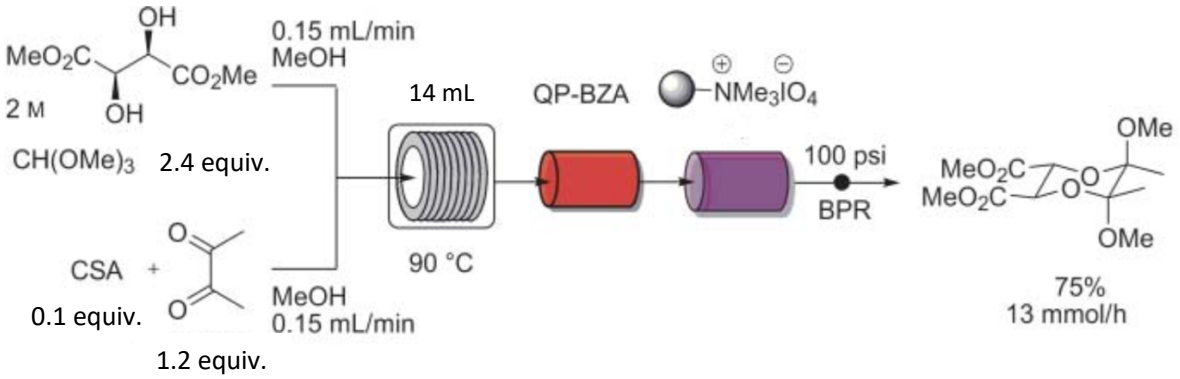
Recently, Ley and colleagues have authored an all-encompassing book chapter that delves into the intricacies of various heterogeneous reagents and scavengers, shedding light on their applications, limitations, and overarching foundational considerations.[24]

Examples of utilizing solid scavengers for sample cleaning steps are abundant. In the following section, significant studies, and examples where scavengers have played a pivotal role as a sample cleaning step will be discussed in three distinct categories: heterogeneous scavenging, homogeneous scavenging, and catch and release strategies. Through this review, the diverse applications, methodologies, and outcomes associated with the utilization of solid scavengers in the purification and sample preparation methods, will be unveiled.

1.4.4. Examples of Heterogeneous Scavenging

In 2010, Carter and his team advanced the field of continuous flow synthesis by employing commercially available flow chemistry microreactors combined with solid-supported reagents and scavengers to create a method for producing butane-2,3-diacetal (BDA) protected derivatives. This technique, which integrates in-line purification systems, has proven exceptionally effective in yielding BDA-protected product with higher efficiency (75%) compared to traditional batch processes (70%). In order to purify the product in-line a column packed with a Quadra-pure benzylamine resin (QP-BZA) was introduced into the

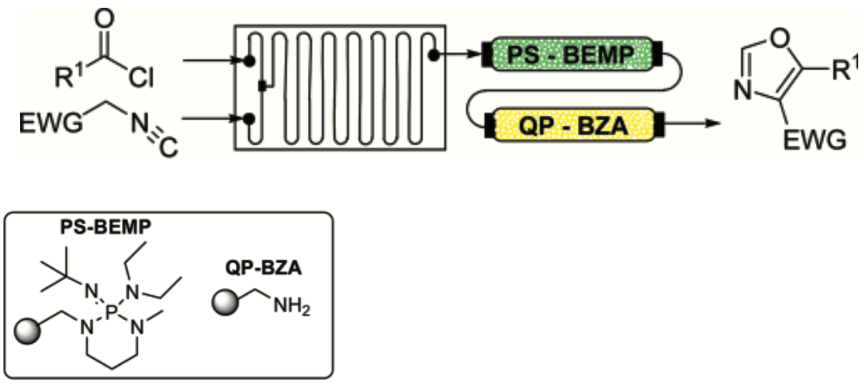
flow stream to scavenge out the acid catalyst, camphor sulfonic acid (CSA) and also to trap out any unreacted butanedione as the imine species. Subsequently, passing the flow stream over periodate resin PS-NMe_3IO_4 removed any unreacted diol (**Scheme 1.2**). After passing the flow stream through these clean-up columns solvent evaporation is the only manual operation required to yield elementally pure BDA tartrate. The outcome highlights the remarkable yields achieved through this approach, reducing the need for labor-intensive manual purification steps.[25]



Scheme 1-2 Flow syntheses of butane-2,3-diacetal tartrate using heterogeneous scavenging for inline purification. (Diagram used in whole from reference [25])

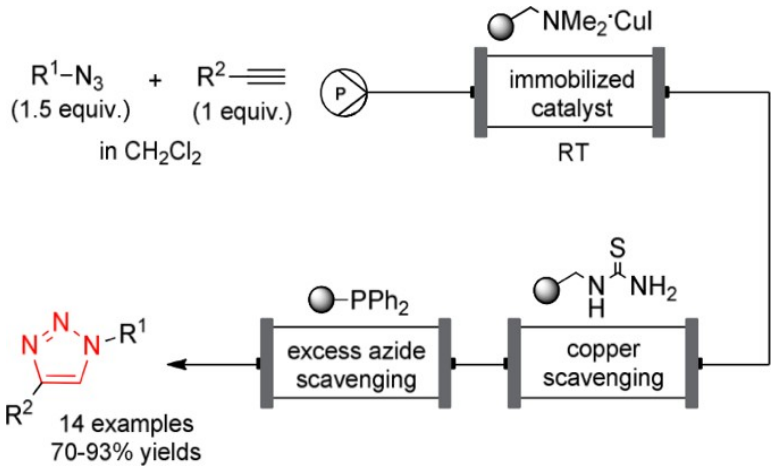
In 2006, Baumann and his team developed an automated meso-fluidic flow reactor for rapid synthesis of oxazole. The synthesis methodology they employed involved the mixing of equimolar quantities of isocyanide and acyl chloride by passing through a glass T-configured chip. This process resulted in the formation of an intermediate addition. A flexible valve selection arrangement directed this reactive flow stream through a packed cartridge of base PS-BEMP (tert-butylimino-2-diethylamino-1,3- dimethylperhydro-1,3,2-diazaphosphorine on polystyrene), leading to a rapid base-catalyzed intramolecular cyclization that yielded the 4,5-disubstituted oxazole as the exclusive product (**Scheme 1.3**). The isolated yield exceeded 80%, with a purity of 90% confirmed by HPLC and 1H NMR. To enhance the final product's purity, they utilized a nucleophilic solid supported scavenger step using the high loading Quadra-pure resin, QP-BZA.

Additionally, they demonstrated the feasibility of automated recycling PS-BEMP columns between runs as part of the reaction sequence.[26]



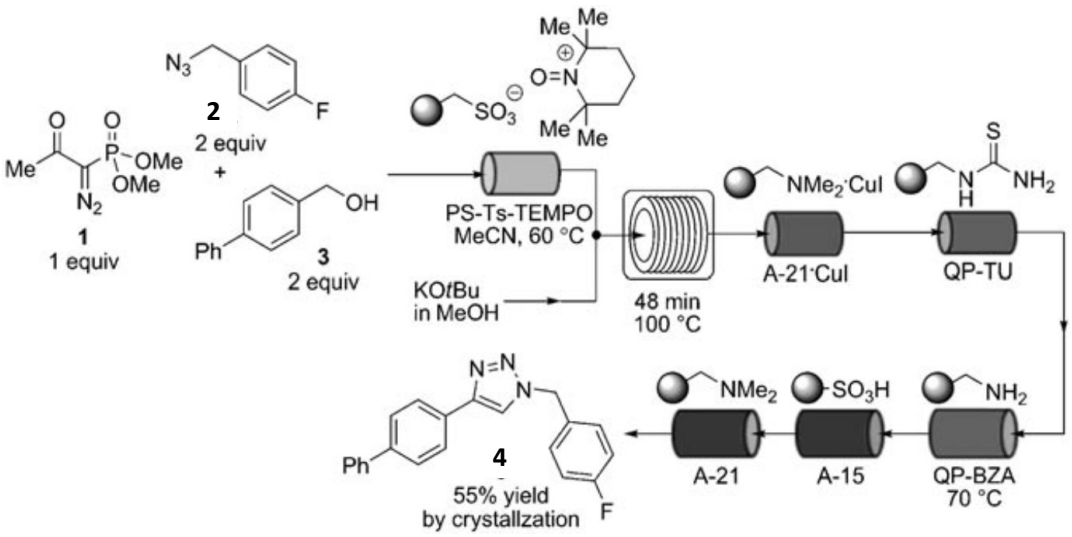
Scheme 1-3 Flow synthesis of 4,5-disubstituted oxazole from isocyanide and acyl chloride using glass T-configured chip mixer, packed column of PS-BEMP for base-catalyzed intramolecular cyclization, and QP-BZA for clean-up the final product. (Diagram used in whole from reference [26])

In the realm of heterogeneous scavenging, Smith and his team made significant contributions in 2007. Their ingenious approach revolved around a modular flow reactor design, fusing a polymer- bound copper catalyst with an array of scavenger resins housed in glass columns. Through strategic immobilization, CuI was bound to a dimethylaminomethyl-grafted polystyrene resin, which not only facilitated the cycloaddition's reactivity but also functioned as a heterogeneous nitrogen base. The incorporation of weak coordinative forces, while necessary, brought the challenge of potential copper catalyst leaching. To counteract this, a thiourea resin was introduced for in-line scavenging, effectively mitigating copper contamination from the solution phase. Employing azide in excess, the reaction proceeded to completion within a single pass at room temperature. Subsequent elimination of unreacted azide was accomplished in-line through a phosphine resin, capturing the azide as an iminophosphorane on the solid phase via a Staudinger reaction (**Scheme 1.4**). This innovative methodology yielded impressive results, including short reaction time, high yields (reaching 93%), without the need for further purification steps; gram-scale production was also successfully implemented.[27]



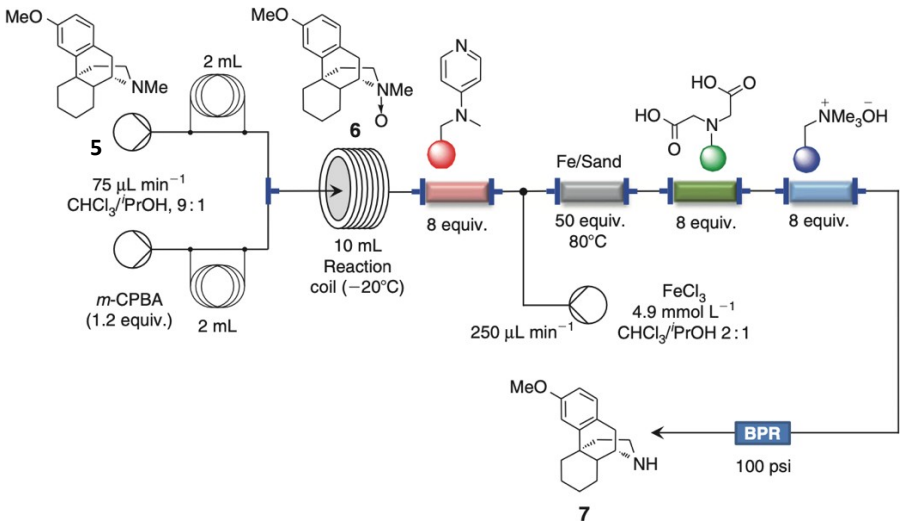
Scheme 1-4 Inline purification using heterogeneous scavenging. (Diagram used in whole from reference [27])

In 2009, Baxendale and colleagues demonstrated a sequential three-step synthetic route yielding triazoles with favorable yields. Notably, they incorporated a series of four scavenger flow tubes (QP-TU, QP-BZA, A-15, A-21) to accomplish product purification and isolation, ultimately achieving the triazole product (**4**) in 55% yield and purity exceeding 95% as confirmed by both ^{1}HNMR spectroscopic and LCMS analyses (**Scheme 1.5**). Of significance, this study highlighted the modular flow reactor's versatility to synergistically engage diverse immobilized reagents and scavenger materials, enabling the synthesis of multicomponent, multistep coupling reactions, thereby circumventing the necessity for interim work-up or manipulation procedures.[28]



Scheme 1-5 Three-step synthesis of a triazole **4** from alcohol **3** using inline heterogeneous scavenging for purification. (Diagram used in whole from reference [28])

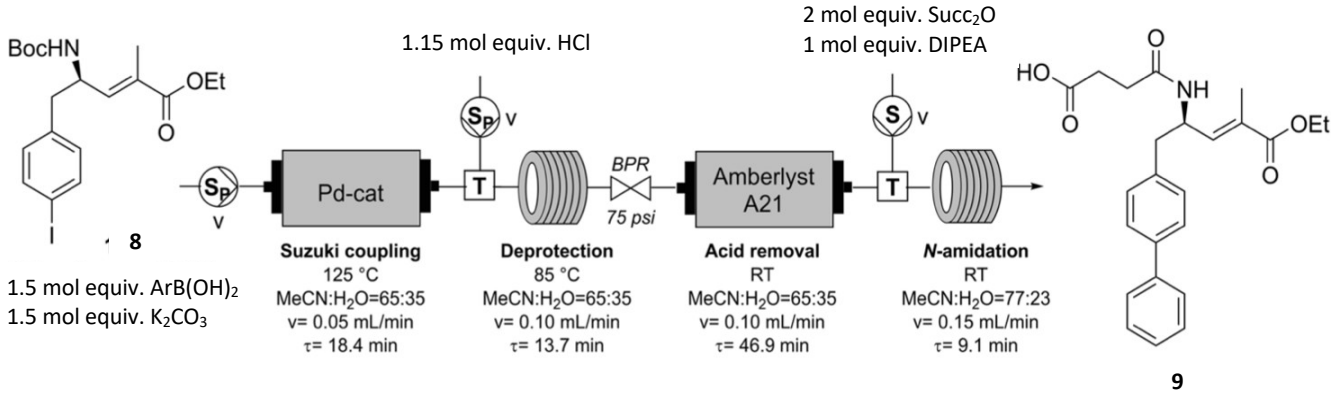
In 2012, Nakano and collaborators conducted an innovative study that demonstrated the efficacy of continuous flow conditions for the N-demethylation of dextromethorphan through the non- classical Polonovski reaction. This process involved two consecutive steps: the initial N-oxidation using m-chloroperbenzoic acid (m-CPBA), followed by iron-catalyzed N-demethylation of the resultant N-oxide. In contrast to traditional batch approaches, this method required fewer unit operations. Batch processes necessitated several steps, such as synthesizing and isolating the N-oxide, subsequent treatment with Fe(0), elimination of Fe salts, crude product isolation, and flash chromatography for purification. Remarkably, Nakano et al. successfully translated this process into a continuous, telescoped framework. This new approach simplified the process to a single unit operation, alongside a simple SiO_2 column purification step, resulting in the production of a pure end product. The study also explored the utilization of cartridges for inline phase separations, which is often employed to eliminate reagents, catalysts, or by-products. This strategy enables the telescoping of multiple reactions before conducting the final purification step offline (**Scheme 1.6**).[29]



Scheme 1-6 Flow reaction schematic for N-demethylation of dextromethorphan 5.

(Diagram used in whole from reference [29])

In 2019, Hiebler and colleagues made notable progress in the field of continuous flow chemistry. They embarked on the development of a complex, multistep-reaction cascade aimed at synthesizing a precursor to sacubitril, an important pharmaceutical compound, using continuous flow techniques. This innovative approach resulted in the successful execution of the synthesis in three consecutive steps, showcasing the efficiency of continuous flow methods for intricate chemical processes. Notably, their methodology also incorporated an interesting in-line purification step. This involved the use of the weakly basic anion exchange resin Amberlyst A21, which effectively adsorbed excess hydrochloric acid and removed various acidic components from the process stream. This purification step was particularly beneficial as it eliminated any hydroiodic acid resulting from the use of an aryl iodide coupling partner in the Suzuki reaction, as well as phenol originating from the oxidation of phenylboronic acid. The absence of significant by-product peaks in the HPLC chromatogram further underscored the success of this in-line purification strategy (**Scheme 1.7**).[30]

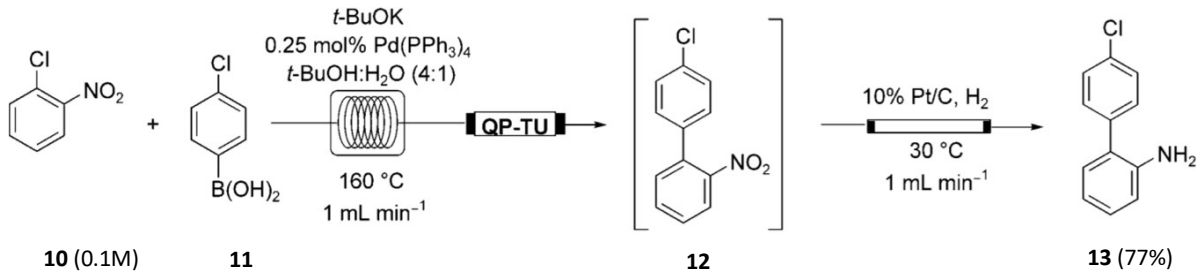


Scheme 1-7 Multistep setup for the synthesis of sacubitril precursor (9) in continuous flow (SP=high-pressure syringe pump, S=syringe pump, T = T- mixer, v = 0.05 mL/min). (Diagram used in whole from reference [30])

In 2007, Hinchcliffe et al. explored the efficacy of Quadra-Pure cartridges as metal scavengers in continuous flow processes. These cartridges demonstrated remarkable efficiency in eliminating trace metal impurities from reaction mixtures. The study showcased five instances where Quadra-Pure cartridges significantly simplified the purification of metal-mediated reactions, outperforming time-consuming conventional methods such as column chromatography, recrystallization, extraction, and distillation. The researchers successfully integrated Quadra-Pure scavenger resins into existing purification setups, effectively removing metal contaminants form reaction products. Notably, these cartridges excelled in removing leached metals and catalytic amounts of homogeneous metal catalysts. This breakthrough ensured the integrity of reactions and product quality in common homogeneous catalytic reactions, such as Suzuki– Miyaura and Sonogashira reactions, and hydrogenations mediated by Wilkinson's catalyst.[31]

Glasnov et al. (2010) introduced a continuous-flow method for synthesizing the crucial intermediate 2-amino-4'-chlorobiphenyl (**13**) used in the production of the fungicide Boscalid. Initially, a high-temperature Suzuki–Miyaura cross-coupling reaction utilizing tetrakis (triphenylphosphine)palladium catalyst facilitated the formation of 4'-chloro-2- nitrobiphenyl (**12**) from 1-chloro-2-nitrobenzene (**10**) and 4-chlorophenylboronic acid (**11**) in a microtubular flow reactor. However, the desired product (**13**) was

contaminated with over-reduced 2-aminobiphenyl due to palladium metal entering the subsequent hydrogenation stage, impacting catalyst selectivity. To address this, a thiourea-based resin (QP-TU) was employed to scavenge palladium metal in-line, effectively minimizing the formation of undesired by-products. Subsequent selective heterogeneous catalytic hydrogenation using platinum-on-charcoal ensured high overall yield and superior selectivity for the desired 2-amino-4'-chlorobiphenyl (**13**), preserving the chlorine functionality. This two-step process, involving both homogeneous and heterogeneous catalysis, with a scavenging step in between, significantly improved the overall yield and product purity, demonstrating the potential of continuous-flow methodologies for complex synthesis with inline purification. (**Scheme 1.8**).[32]

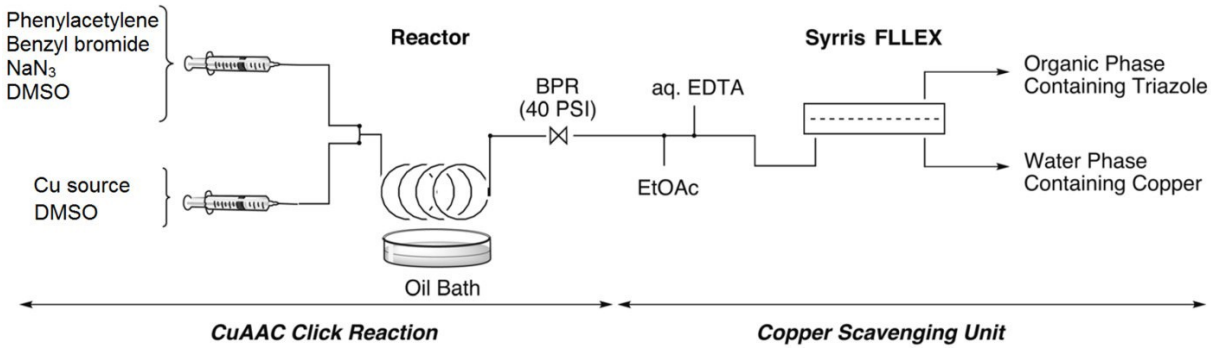


Scheme 1-8 Schematic diagram and reaction conditions for the two-step continuous-flow synthesis of 2-amino-4′-chlorobiphenyl (**13**). (Diagram used in whole from reference [32])

1.4.5. Example of Homogeneous Scavenging

In the realm of homogeneous scavenging, a significant advancement was presented by Vural and co-workers in 2014. They addressed the challenge of efficiently removing metal traces in catalytic chemistry processes. While scavenging columns effectively eliminate metal from product streams, they require replacement upon saturation, disrupting continuous operations. Although these columns are suitable for small-scale production, they are unsuitable for larger-scale experiments. Vural and team ingeniously combined

scavenging and phase separation within a single unit, streamlining the process. By employing liquid-liquid extraction and a porous polytetrafluoroethylene membrane, they achieved uninterrupted metal scavenging. This unit was coupled downstream of a copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, offering continuous metal removal. A Syrris Flow Liquid-Liquid Extraction (FLLEX) module facilitated separation of the organic product phase and aqueous catalyst phase (**Scheme 1.9**). Through process parameters optimization, the 1,2,3 triazole product (which is used in variety of applications from drug discovery and development to material science and chemical biology), was obtained with a high yield of 92% and met pharmaceutical purity requirements (copper content of triazole product below the limit for APIs of 15 ppm) in a single extraction step. This innovative approach significantly enhances metal catalyst removal efficiency in continuous-flow chemistry, providing a practical solution for large-scale synthesis.[33]

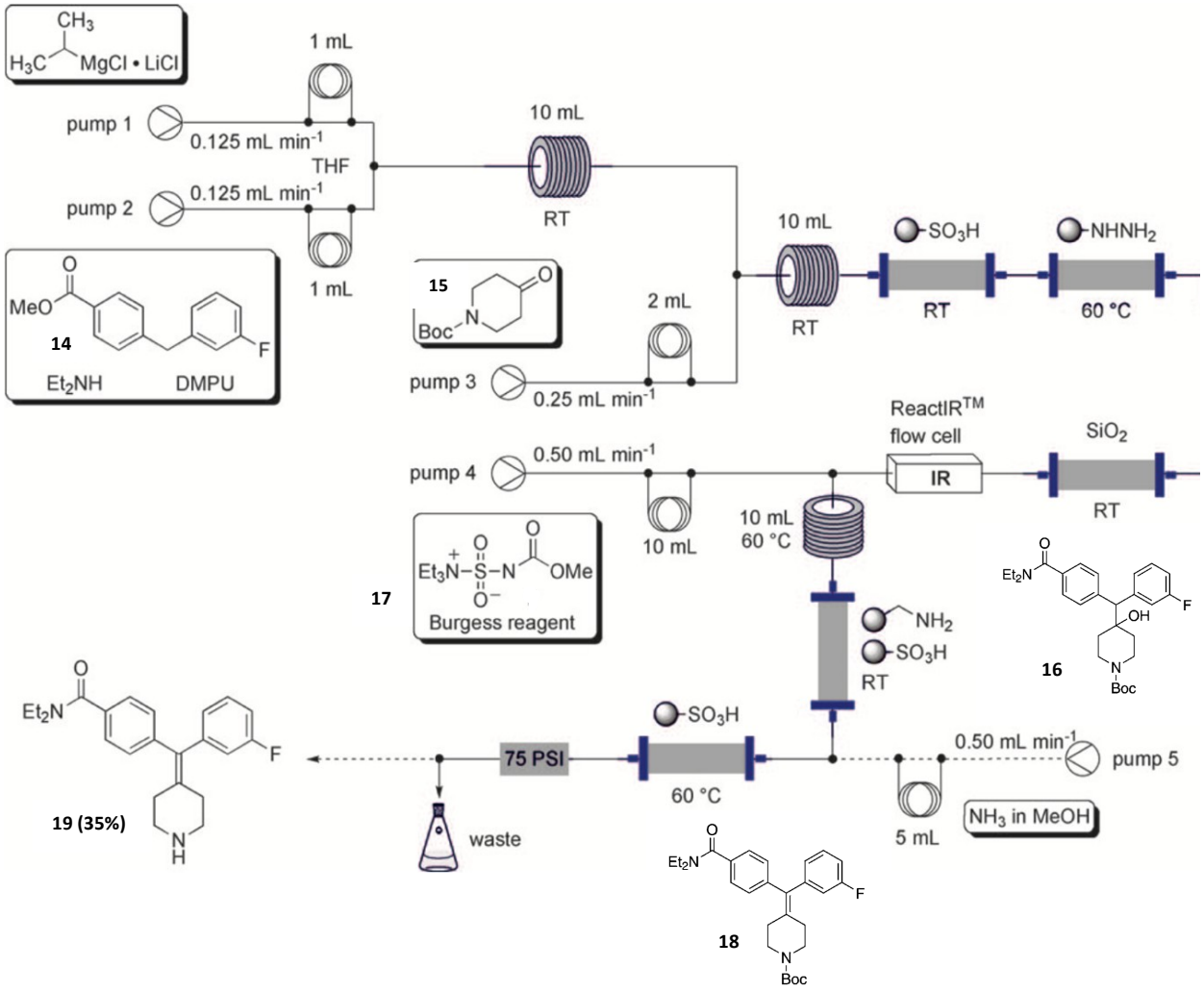


Scheme 1-9 Continuous flow setup of one-pot CuAAC click reaction coupled with metal scavenging unit. (Diagram used in whole from reference [33])

1.4.6. Examples of Catch and Release Strategy

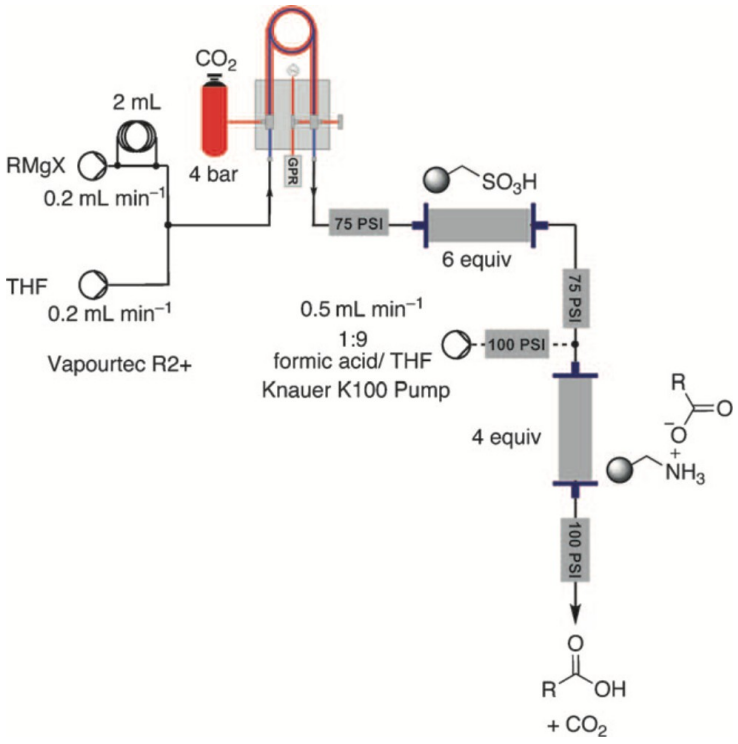
An interesting approach regarding the use of resins is their application in catch and release strategies where the product is temporarily trapped in a cartridge and then released in pure form using other conditions.

A notable example of this strategy can be found in the work of Qian and colleagues in 2010. They successfully developed a four-step continuous flow synthesis approach for a potent d-opioid receptor agonist **19**. To achieve this, they employed a combination of pumping devices and cartridges pre-loaded with precisely chosen reagents or scavengers to ensure the clean delivery of the desired product (**Scheme 1.10**). One of these scavengers was quadra-pure sulfonic acid (QP-SA), a resin presenting sulfonic acid (–SO3H) groups, making it proficient at sequestering amines. The exit stream emerging from the third reactor (the last in the series, **Scheme 1.10**) contained the Boc-protected **18**. This stream was then directed through a heated column packed with QP-SA. This column was to deprotect the compound and to capture the target molecule efficiently. Any unreacted amide from the first two steps was simply pumped to waste, while all key intermediate **16** had already been cleanly converted to the desired product **19**. Elution of the acidic column using a solution of NH_3 in MeOH, facilitated product release and completed the synthesis in a continuous fashion and gave the **19** in 35% overall yield and in high purity over the four steps.[34]



Scheme 1-10 Four step continuous flow synthesis of N,N-diethyl-4-(3-fluorophenylpiperidin-4-ylidenemethyl)-benzamide (**19**) using catch and release strategy as the purification step. (Diagram used in whole from reference [34]).

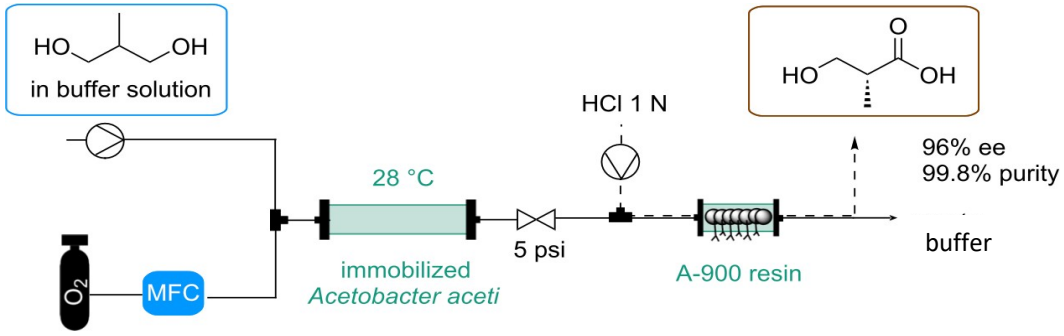
Polyzos and colleagues in their 2011 study, designed a continuous-flow synthesis method for carboxylic acids. In their setup, they introduced a two-step in-line work-up and purification process using polymer-supported reagents (**Scheme 1.11**). Firstly, the exiting reaction stream was directed through a glass cartridge containing polymer-supported sulfonic acid (QP-SA), which removed magnesium salts and converted carboxylates into acids. Subsequently, the stream passed through a second cartridge with polymer-supported ammonium hydroxide (A-900) in a "catch-and-release" protocol, effectively trapping the acids. After a THF wash to eliminate impurities, an external pump introduced a third stream to release the carboxylic acids from the A-900 resin, using a formic acid/THF solution (1:9). This approach streamlined carboxylic acid synthesis and purification in continuous-flow systems, demonstrating the potential of catch-and-release strategies to enhance the efficiency and productivity of chemical processes.[35]



Scheme 1-11 Flow reactor configuration for the carboxylation of Grignard reagents.

(Diagram used in whole from reference [35])

The application of catch-and-release strategies in continuous synthesis has proven highly effective for both enhancing efficiency and recycling of starting materials. This approach is particularly valuable in the efficient production of essential building blocks like nucleoside derivatives and L-pipecolic acid. In a study by Vitis et al. In 2019, they achieved high efficiency by implementing straightforward trapping columns downstream of the biocatalytic process. These columns effectively separated the pure products from the mixture and facilitated the recirculation of the aqueous phase in a closed-loop system. **Scheme 1.12** illustrates the production of optically pure alcohols through this approach. A pivotal step in this process was in-line purification, which featured the use of ion exchange Ambersep 900 OH- resin. This resin exhibited remarkable efficiency by capturing quantitatively the acid present in the outflowing stream (>99.8% pure by NMR, ee 96%) after elution from the resin with a 1 N HCl solution.[36] This exemplifies the application of catch-and-release strategies ultimately leading to the recycling of starting materials and the production of high-purity chemical compounds.



Scheme 1-12 Exemplar catch and release purification of a stereoselective oxidation.

(Diagram used in whole from reference [52])

The choice of sample preparation technique is not arbitrary; it is guided by the specific analytical goals, desired detection limits, and the inherent characteristics of the target analytes. A well- chosen technique can significantly improve the efficiency of the overall analysis, leading to accurate and reliable results. However, the initial step of an analysis procedure—sample preparation—often proves time-consuming, presenting a significant bottleneck in the analytical workflow. Herein lies the importance of automation.

Automation in sample preparation not only addresses the challenge of time consumption but also introduces a host of other benefits. Advancements in automation technology have streamlined the sample preparation process, mitigating human error and drastically increasing throughput. In the next section, we delve into the nuanced benefits and limitations of automation in sample preparation, elucidating its role in modern analytical chemistry.

1.5. Automation in Sample Preparation for GC Analysis

Even with the remarkable improvements in analytical instrumentation over the past seventy years, the pursuit of advanced analytical techniques that prioritize efficiency, environmental sustainability, and ease of use remains a crucial demand in the modern analytical chemistry.[37] The challenge lies in addressing the time-consuming aspects of sample preparation, which are essential for ensuring accurate and reproducible results.[38] Pre-analysis sample preparation and extraction can be the most time-consuming part of an assay (**Figure 1.3**). As analytical run times becoming shorter and shorter (few minutes in LC-MS and even shorter in GC-MS, depending on parameters used for analytical method), multiple, laborious steps make sample preparation the bottleneck. Moreover, the most significant sources of errors in chromatographic analyses are from sample processing (30 %) and operator error (19 %; **Figure 1.4**),[39] suggesting the need to improve or remove these aspects. The demand for high-throughput sample preparation systems, integrated seamlessly with analytical instruments, will increases during the years ahead.[40] In this context, automation emerges as the best strategy to enhance laboratory productivity.[41]

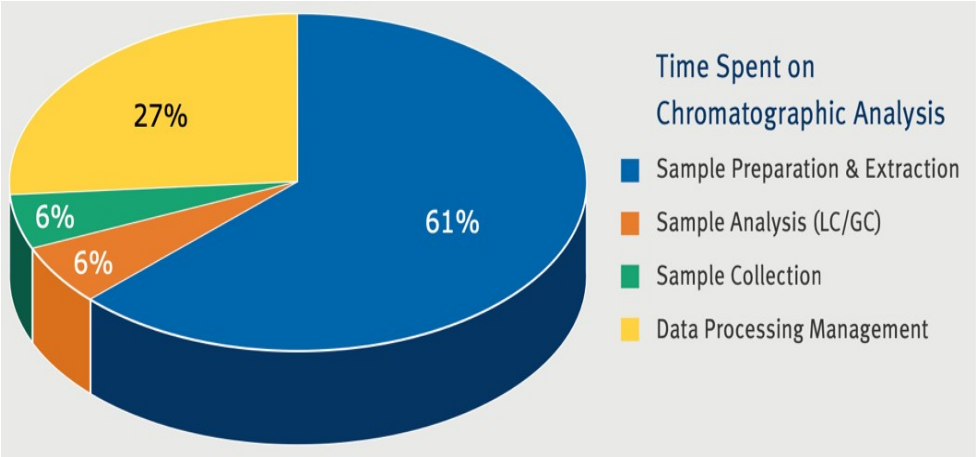


Figure 1.3 Survey results for the distribution of time that analytical chemists spend on sample analysis. (Diagram used in whole from reference [39])

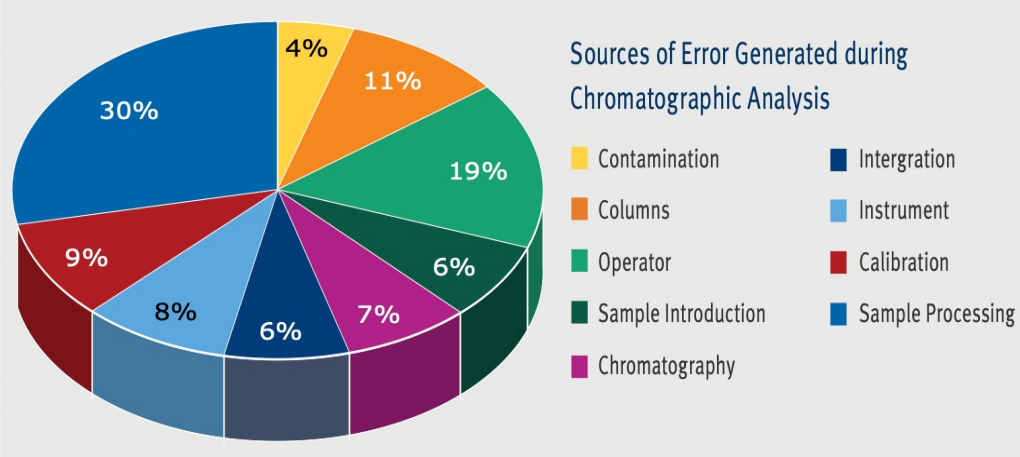


Figure 1.4 Survey results for the distribution of errors generated during sample analysis. (Diagram used in whole from reference [39])

1.5.1. Enhancing Efficiency and Throughput

Automation offers a transformative solution to the challenges posed by manual sample preparation. By automating sample preparation steps, laboratories can significantly

accelerate processing times, especially when time-consuming projects like bioequivalence studies require the processing of hundreds of samples.[42] For instance, a recent study demonstrated a remarkable 33% reduction in solid-phase extraction processing time for 96 samples compared to manual processing.[43]

This advantage has been made possible by utilizing the concept of automated parallel–sample processing; automated parallel processing solid-phase extraction was introduced and demonstrated to be practical a decade ago.[44] Under this method, numerous samples are extracted simultaneously. Although the equipment requirements for parallel processing can be more specialized, or at least more cleverly designed, the great pay back occurs in terms of dramatically improved throughput. With early, automated systems, individual samples were processed in series,[45] where the next sample in the series was not started until the preceding one had been completed (or was well on its way). With serial sample processing, automated solid-phase extraction systems were slower than manual systems, but because they could operate continuously around the clock, timesaving was still achieved.[46],[47]

Time saved on routine sample preparation is time that analysts can spend on more rewarding tasks, such as method development or data interpretation, and such tasks enable analysts to add the most value to the entire analysis process.[44]

1.5.2. Precision and Consistency

The numerous manual handling steps involved in sample preparation and extraction processes are inherently susceptible to errors and can introduce unwanted variability into analytical results. This inherent variability is a persistent challenge that impacts the reliability and robustness of analytical processes.[48]

The process of preparing a sample for GC analysis may include different manipulations such as addition, dilution, concentration, and dissolution, all aimed at optimizing analytes performance in the GC system, thus enhancing the overall chromatographic process and detection. Each of these manipulations, if performed manually, introduces a series of unwanted variabilities, which, when automated, offer an assurance of higher precision and

accuracy in the results.[49] Automation effectively standardizes these steps by providing precise control over parameters such as volume, extraction time, and temperature, thus reducing the potential for variability across samples and thereby ensuring reproducibility and enhancing the overall reliability of the analysis.

Moreover, automated sample preparation goes a step further by eliminating much of the variability in results attributable to different operators. In scenarios where a single method is implemented across multiple laboratories, operator technique can introduce significant variations. However, automated systems, guided by a consistent set of parameters and protocols, eliminate this source of variability, resulting in data quality that is more uniform and consistent from one site to another.[50]

1.5.3. Economical and Sustainable Practices

Automation contributes to cost savings through optimized resource utilization. Reducing or eliminating the amount of labor spent on each sample is often where the biggest savings can be made, however, costs also come down when expensive analytical instruments perform more analyses per unit time. With enhanced throughput and efficient use of skilled staff, the cost per sample is reduced. Moreover, automation systems sometimes can optimize the usage of reagents which are expensive to buy, store and to dispose of. Automation often incorporates sensors and monitoring devices that collect real-time data on the progress of a process. This data can be analyzed to optimize solvent usage. If the system detects that a smaller amount of solvent is sufficient for a particular task, it can adjust parameters accordingly, minimizing excess use. Miniaturization of the method such as the use of microfluidics, is a possible part of the automation bundle. By working with smaller volumes in microscale processes, less solvent is needed overall, and the costs associated with the purchase and disposal of reagents is also lower.

Additionally, many automated processes can operate within closed systems or containment units. This not only prevents solvent exposure to the external environment but also reduces evaporation, supporting greener practices.[39], [50]

1.5.4. Improved safety

Certain samples contain hazardous or toxic compounds, posing risks to operators during manual handling. Automation eliminates or significantly reduces human interaction with these substances, ensuring the safety of laboratory personnel.[50]

1.5.5. Challenges and Limitations of Automation in Sample Preparation

While automation brings many benefits to sample preparation, it's essential to recognize its limitations:

1. Liquid Level Sensing: Many modern automated systems use liquid level sensing mechanisms to determine the presence or absence of liquid in samples. By using the presence or absence of electrical conductance between different areas on the liquid transfer tips, liquid level sensing can detect whether or not liquid, in a suitable form for transfer, is present. If liquid is not present, the transfer tip can be repositioned to reattempt the transfer. This feature is particularly valuable when dealing with dirty or biological samples that may contain clots, flocculant or other inhomogeneities. In some cases, the automated system can be programmed to try multiple times to get a suitable sample. if a suitable sampling of liquid cannot be obtained, the workstation will skip the sample. However, it's important to note that liquid level sensing is not always as reliable as human operators who can adapt to unexpected issues. In some cases, manual intervention may be more effective in handling challenging samples.[44]

2. Sample Stability: One significant limitation arises when dealing with samples that are not chemically or physically stable over time. The challenge becomes more problematic when utilizing the serial-processing algorithm with unstable samples. Consider, for instance, determining a drug substance in plasma where the

processing time per sample is 3 minutes, and 60 samples are to be processed sequentially. In such cases, it becomes crucial to ascertain the stability of the drug over the 3-hours processing duration. Furthermore, the physical stability of the plasma becomes a critical factor. If protein flocculation initiates due to denaturization, the likelihood of clogging in solid-phase cartridges or wells rises significantly (clots, flocculant and other inhomogeneities precipitate and block the flow), leading to high extraction failure rates towards the end of the run. Similar challenges can occur when dealing with food or other biological samples. Parallel processing approaches are less affected by sample stability issues because they process samples quickly (between 10 to 20 minutes), which reduces the chances of instability.[44]

3. Initial Installation Effort: Setting up an automation system in a laboratory can be labor- intensive. Skilled staff are required to ensure the system operates efficiently over an extended period. While robots may not process samples faster than human technicians (in fact, they may be slower), their advantage lies in their ability to work 24 hours a day without rest.[47]

Recognizing these above limitations, while using automation thoughtfully can maximize its benefits in the laboratory.

1.6. In This Study

While gas chromatography continues to gain prominence as a powerful analytical tool, the issue of column contamination remains a significant concern. Contaminants typically fall into two categories: 1) nonvolatile and 2) semi volatile residues. Nonvolatile residues don't elute and coat the column through accumulation over time, interfering with solute partitioning and potentially interacting with active solutes, causing issues with peak shape. Semi volatile residues accumulate and elute over hours to days, leading to problems with peak shape and size and issues relating to baseline consistency.

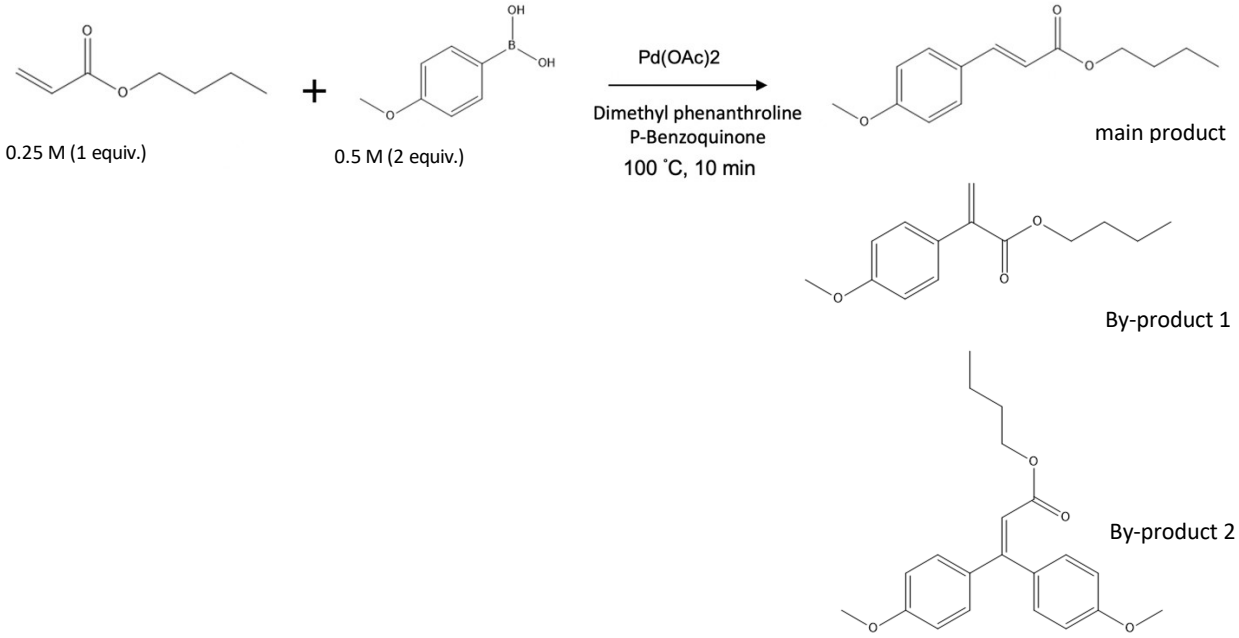
Contaminants mainly stem from injected samples, especially those from complex matrices like biological fluids and soils. Rigorous extraction procedures can't always prevent small amounts of contaminants from accumulating over several injections, particularly with certain injection techniques such as on-column injection where a liquid sample is introduced directly onto the chromatographic column without prior vaporization (This technique is particularly valuable for samples sensitive to thermal degradation). Minimizing these residues is key to reducing contamination problems, but their presence and identity are often unknown. Here, we propose an innovative solution using solid scavengers for automated sample preparation. These versatile materials can selectively interact with specific analytes, effectively removing harmful compounds from reaction mixtures before they enter the column, enhancing the efficiency and reliability of gas chromatography.

1.6.1. Research Objectives

Obtaining pure product isolation through exclusive utilization of inline heterogeneous cartridges demands a sequence of distinct scavengers and immobilized reagents. Yet, the maintenance of these columns can be problematic, susceptible to fouling, necessitating replacement or regeneration during upscaling, which can prove costly and labor-intensive. Furthermore, it's imperative to note that the efficiency and functionality of a given quantity of scavenger can be constrained when employed in an immobilized packed format where

particles are fixed in their positions, as opposed to its more efficient free-powdered state. The core concept underpinning this research is the utilization of scavengers in their free-powdered form, integrated into an automated process facilitated by a robotic arm.

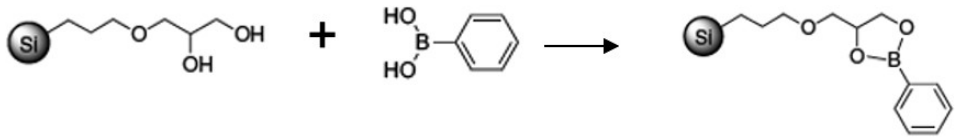
In this study, we selected an Oxidative Heck reaction, a significant carbon-carbon cross-coupling reaction as our model reaction. The reaction occurs between Butyl acrylate and 4-Methoxyphenyl Boronic acid in the presence of Palladium acetate as catalyst, Dimethyl phenanthroline as ligand and para-Benzoquinone as oxidant (**Scheme1.13**).



Scheme 1-13 Base-Free Oxidative Heck Arylations of n-Butyl Acrylate. Palladium acetate ($Pd(OAc)_2$), Dimethyl phenanthroline (dmphen), and para-Benzoquinone (p-Benzoquinone) are used as catalyst, ligand and oxidant respectively. [51]

In the oxidative Heck reaction, the presence of 4-Methoxyphenylboronic acid in the reaction mixture posed challenges for subsequent gas chromatography analysis due to its potential detrimental effects on the chromatography column. Phenylboronic acid tends to interact with the stationary phase of the column, leading to column degradation and decreased quality of chromatographic analysis. To overcome this issue, phenylboronic acid must be removed from the reaction mixture before GC injection. [53],[54]

The idea is to use solid scavengers in free-powder (suspended) form in the sample preparation process to selectively remove phenylboronic acid from the reaction mixture. The selection of an appropriate solid scavenger is critical to ensure the efficient and selective removal of phenylboronic acid while preserving the integrity of the desired reaction product. SiliaBondDiol resin, produced by Silicycle inc. is a silica-based solid-phase scavenger functionalized with diol groups exhibits a strong affinity for boronic acids, making the resin well-suited for scavenging phenylboronic acid from the reaction mixture (**Scheme 1.14**). This resin was selected as our scavenger of choice for this study.



Scheme 1-14 Phenylboronic acid scavenging mechanism with SiliaBoundDiol scavenger.

Development of an Automated Sample Preparation Method:

The primary objective of this research is to develop an automated sample preparation method that effectively removes harmful compounds from the sample matrix, which with the chemistry of interest will focus on phenylboronic acid removal.

The method includes three main steps:

- First is transferring specific amount of a stock solution containing known concentration of phenyl boronic acid and Internal standard into a pre-filled vial of scavenger.
- Next is using the micro-syringe itself as a mixing module for mixing the phenylboronic acid with scavenger by aspiration and dispensing the solution.
- Last is removing the top transparent portion of mixture which is free of any suspended solid particles for further analysis.

All these liquid transfers and syringe movements are controlled with a robot arm and through different parameters such as transfer volumes, aspiration/dispensing volume,

aspiration/dispensing flowrates, syringe position in vials (penetration depth), number of aspiration/dispensing cycles in mixing step, and waiting times for particles settling at the bottom of vials.

Optimization of Extraction Conditions:

The research aims to optimize the extraction conditions for the automated sample preparation method. Factors such as solvent selection, extraction time, mixing intensity, and scavenger equivalents will be studied to achieve maximum extraction efficiency and reproducibility.

2. Results and Discussion

2.1. Primary Manual Experiments

The primary manual experiments were essential in laying the groundwork for optimizing the scavenger conditions for phenylboronic acid removal from the reaction mixture. These experiments allowed us to gain valuable insights into the scavenger's behavior and its effectiveness in scavenging the target compound. The detailed analysis of each experiment and its significance in the overall optimization process in discussed in this section.

2.1.1. Experiment 1: Determining Initial Scavenger Amount

To begin, we aimed to determine the appropriate amount of Diol scavenger required to efficiently remove phenylboronic acid from the reaction mixture. In this experiment, four vials were prepared with varying amounts of scavenger to observe its impact on scavenging efficiency. Vial#1 contained 1 mL of a standard solution consisting of 0.25 M phenylboronic acid in dimethylacetamide (DMA). To track the scavenging efficiency accurately, an internal standard (toluene) was added to Vial#1 at an equivalent molar ratio with respect to phenylboronic acid. Vial#2, Vial#3, and Vial#4 were prepared with 1 equivalent (molar ratio with respect to phenylboronic caid), 2 equivalents, and 3 equivalents of Diol scavenger, respectively.

The vials, except for Vial#1, were placed on a stirrer plate and stirred for one hour. However, after stirring, we observed that the solutions in the vials were cloudy, likely due to the presence of scavenger particles (suspended particles). To address this, the vials were left undisturbed for 30 minutes to allow the solid scavenger particles to settle out (**Figure 2.1**). Following this, 50 μL of the top transparent liquid from each vial was removed and diluted with 500 μL of dmso-d6 for 1HNMR spectroscopic analysis.

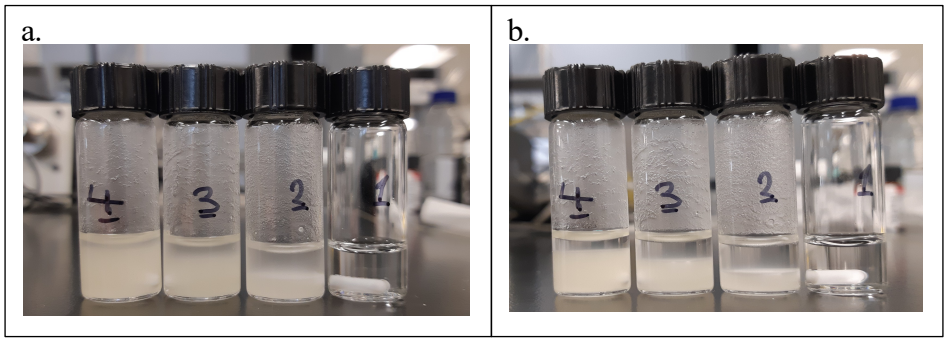


Figure 2.1 appearance of the mixtures in vial#1, vial#2, vial#3, and vial#4 containing standard solution with 0,1,2, and 3 equivalents of Diol scavenger with respect to methoxyphenyl boronic acid. **a.** Right after stirring; the solution is cloudy because of solid particles suspension. **b.** After 30 minutes leaving the vials undisturbed.

The results were compared between Vial#1 (standard solution) and the scavenger-containing vials (Vial#2, Vial#3, and Vial#4) and the scavenging efficiency was determined based on the decrease in the phenylboronic acid peak intensity in the ^{1}HNMR spectra (sample calculation provided in section 4.6.4).

The results showed that Vial#2 achieved 36% removal, Vial#3 achieved 55% removal, and Vial#4 achieved 65% removal of phenylboronic acid (**Table 2.1**). While the 65% efficiency in Vial#4 was an improvement, it fell short of our desired removal level (100%). Attempting to increase the scavenger beyond 3 equivalents led to the solution becoming too dense, transitioning from a liquid to a muddy, dense texture (no room in the liquid environment for solid particles to move around), which is not conducive to efficient mixing or sample preparation.

Table 2-1 Result of Experiment 1, each vial contains 1 mL solution of 0.25 M 4-methoxyphenyl boronic acid in DMA and 1 equiv. Toluene as internal standard but different amount of scavenger. All vials stirred for 1 hr.

Entry	vial	# of equivalents of diol resin	Boronic acid removal
1	Vial#2	1	36%
2	Vial#3	2	55%
3	Vial#4	3	65%

2.1.2. Experiment 2: Dilution Effect with Dichloromethane (DCM)

To enhance scavenging efficiency, we introduced DCM as a solvent to dilute the solution, creating more room for scavenger particles to move around in the liquid environment. A vial was prepared by adding 50 μL of the standard solution (from Vial#1) to 950 μL of DCM and 3 equiv. of Diol scavenger.

In this experiment, we employed a shaking method to mix the solution – rather than stirring that appears to crush the scavenger and create fines. The vial was shaken up and down 20 times at 5 min. intervals over the course of an hour. Following the shaking process, the vial was left undisturbed for 10 min. to facilitate solid particle precipitation. The top transparent solution was then removed and mixed with dmso-d6 for ^{1}HNMR spectroscopic analysis. The comparison between standard solution and this sample revealed an impressive 77% removal of phenylboronic acid. The dilution effect of DCM played a significant role in enhancing scavenger efficiency. By diluting the solution, we may have created improved access to the active sites on the scavenger, leading to improved removal efficiency.

2.1.3. Experiment 3: Introducing Manual Mixing with Syringe

To further develop the initial parameters of the robotic system, we decided to employ a small syringe with a needle for manual mixing. Next solution was prepared with the following composition:

- 50 μL of standard solution
- 950 μL of DCM
- 6 equiv. of diol scavenger

For manual mixing, the syringe was manually filled with 0.5 mL of the top transparent liquid and emptied back into the solution. This process was repeated 15 times, with a 60-second waiting time between each emptying and its next filling step. This waiting time allowed solid particles to settle to prevent needle clogging. ^{1}HNMR spectroscopic analysis was performed after 10 minutes from the last mixing process. The comparison between standard solution and this sample showed 77% phenylboronic acid removal. This

experiment provided valuable insights, indicating that increasing the scavenger amount from 3 to 6 equiv., even with a decreased contact time (~15 min in comparison to 1 h), led to the same efficiency. This finding confirmed that scavenger efficiency could be enhanced by increasing the scavenger amount.

2.1.4. Experiment 4: Effect of Increasing Scavenger Amount

Building upon the findings of Experiment 3, we further investigated the effect of increasing the scavenger amount. A solution with the following composition was prepared:

- 50 μL of standard solution
- 950 μL of DCM
- 12 equiv. of Diol scavenger

All other conditions were kept the same as Experiment 3. The ^{1}HNMR spectral analysis of this experiment compared to the standard solution demonstrated an impressive 91% phenylboronic acid removal. This result was a significant improvement, but it was still not sufficient for achieving our desired level of removal.

2.1.5. Experiment 5: Effect of Increasing Aspiration-Dispensing Cycles

We were curious about what if we increase the number of mixing cycles (increased contact time)? To evaluate the effect of increased contact time on efficiency of phenylboronic acid removal, the below solution was prepared:

- 50 μL of standard solution
- 950 μL of DCM
- 12 equiv. of Diol scavenger

In this experiment, the number of aspiration-dispensing cycles was doubled (30 times, instead of 15 times) compared to Experiment 4. Analysis of this mixture showed no improvement in scavenger efficiency, maintaining the same 91% removal as Experiment 4. This finding indicated that the maximum scavenger efficiency for 12 equiv. of scavenger had been reached, and further increasing the number of aspiration-dispensing cycles will not further enhance scavenging efficiency. Summary of the conditions and result of manual

experiments are listed in **Table 2.2**.

Table 2-2 Summary of the conditions and results of manual experiments. Experiment 1 is the comparison between 1,2, and 3 equiv. of scavenger in 1 mL of DMA as the solvent and 1 h stirring (entry 1,2, and 3). Experiment 2 shows the dilution effect of DCM on scavenger efficiency (entry 4). Experiment 3,4, and 5 is the comparison of scavenger amount (entry 5 and 6) and number of mixing cycles (entry 6 and 7).

Entry	Exp.#	Diol scavenger amount (equiv.)	Diol scavenger amount (mg)	solvent	Mixing method	Waiting time for precipitation	Phenylboronic acid removal
1	Exp.1	1	219	1 mL DMA	1hr stirring	30 min	36%
2	Exp.1	2	438	1 mL DMA	1hr stirring	30 min	55%
3	Exp.1	3	657	1 mL DMA	1hr stirring	30 min	65%
4	Exp.2	3	32.9	50 µL DMA 950 µL DCM	20 handshaking 5 min. interval	10 min	77%
5	Exp.3	6	65.8	50 µL DMA 950 µL DCM	15 manual aspiration-dispensing 1 min. interval	10 min	77%
6	Exp.4	12	131.5	50 µL DMA 950 µL DCM	15 manual aspiration-dispensing 1 min. interval	10 min	91%
7	Exp.5	12	131.5	50 µL DMA 950 µL DCM	30 manual aspiration-dispensing 1 min. interval	10 min	91%

2.1.6. Experiment 6: Scavenger Selectivity

Apart from optimizing scavenger efficiency, it was crucial to examine the scavenger's selectivity, ensuring that it only targets phenylboronic acid while remaining inert to other mixture components, such as starting materials and reaction product and byproducts. In Experiment 6, we assessed the scavenger's effect on other compounds in the mixture.

^{1}HNMR spectroscopic analysis was performed on n-butyl acrylate (the starting material), the main product, and two byproducts in the presence of the scavenger, with the conditions identical to Experiment 4. The results showed that the diol scavenger had no scavenging effect on these compounds and all of them remained intact after scavenging process (same concentration before and after scavenging process). This demonstrated the high selectivity of the scavenger towards phenylboronic acid, making it an ideal choice for our purpose

(**Table 2.3**).

Table 2-3 Result of the effect of scavenging process on other compounds of the reaction mixture in the absence (entry 1,2,3, and 4) and in the presence of phenyl boronic acid (entry 5 and 6). All solutions were prepared with 50 μL 0.25 M of desired molecule in DMA and 950 μL DCM, 15 times aspirating-dispensing with 1 min interval.

Molecule	Amount of 4-Methoxyphenyl boronic acid in the solution	Removal of desired molecule
1 equiv. product	0	0
1 equiv. butyl acrylate	0	0
1 equiv. by-product 1	0	0
1 equiv. by-product 2	0	0
1 equiv. product	1 equiv.	0
1 equiv. butyl acrylate	1 equiv.	0

In summary, the primary manual experiments were helpful in determining the initial guess for parameters and conditions used in robotic experiments. By diluting the solution with DCM and increasing the scavenger amount to 12 equiv. with respect to phenyl boronic acid, we achieved an impressive 91% scavenger efficiency. Additionally, the selectivity of the scavenger ensured that other components in the reaction mixture remained unaffected.

The results of these manual experiments served as a solid foundation for the implementation of robotics. The condition established in Experiment 4, with 12 equivalents of scavenger and 15 aspiration-dispensing cycles, provided a valuable starting point for the automated sample preparation process.

2.2. Robotic Experiments

2.2.1. Results of Experiments with DMA (Dimethylacetamide) as the Solvent for Standard Solution

In the initial phase of the robotic experiments (Experiment 7), parameters obtained from our manual experiments were adopted, which involved the use of 12 equiv. of scavenger and mixing with 15 aspiration-dispensing cycles. Following each dispensing process, solid particles were observed to settle rapidly within a few seconds, leading to a reduction in the waiting time between each dispensing and the subsequent aspiration from 60 to 10 sec. The rationale behind this modification was rooted in the limited interaction between the settled particles and the phenylboronic acid in the solution. This change was expected to have minimal impact on the overall efficiency. However, despite the expectation of achieving an efficiency of 91%, similar to the manual experiment conducted under these conditions, the initial robotic experiment (Experiment 7) yielded a lower efficiency of only 71-73%. The reduced efficiency was attributed to the limitation in the dispensing flow rate of the 1 mL syringe on the robot. The maximum applicable dispensing flow rate of 100 μL per sec. may have limited solution mixing.

To compensate for the lower mixing intensity in Experiment 7, one approach was to increase the number of aspiration-dispensing cycles in the subsequent Experiment 8. By performing 30 aspiration- dispensing cycles instead of 15, an attempt was made to improve the mixing efficiency and potentially increase the scavenger performance. However, Experiment 8 showed only a marginal improvement, with the efficiency reaching 75%.

Recognizing that increasing the number of aspiration-dispensing cycles was not a viable option due to the associated prolonged processing time, exploration of other avenues to enhance scavenger performance commenced. For Experiment 9, the use of 15 equiv. of scavenger and 15 aspiration-dispensing cycles was examined. To avoid the needle tip being

in close proximity to the settled scavenger, the milligrams of scavenger remained unchanged, but the volumes of the standard solution and DCM were adjusted. Specifically, the standard solution volume was reduced to 40 μL (from the original 50 μL), and the DCM volume was increased to 960 μL (from the original 950 μL) to maintain a total volume of 1mL. These changes resulted in an increase to 82% extraction of the Phenylboronic acid, which, although an improvement, still fell short of the desired efficiency.

In an attempt to further enhance scavenger performance, Experiment 10 was conducted with a greater ratio of scavenger (20 equiv.) to phenylboronic acid, while keeping the number of aspiration-dispensing cycles at 15. Similar to Experiment 9, adjustments in the standard solution volume (30 μL) and DCM volume (970 μL) were made to maintain a total volume of 1mL. Experiment 10 demonstrated 86% phenylboronic acid removal, indicating that an increase in scavenger equivalents did lead to some improvement, albeit still not enough to meet the desired efficiency. A summary of the parameters and results of Experiment 7-10 provided in **Table 2.4**.

Table 2-4 Results of scavenger efficiency in Robotic experiments (Exp. 7-10) using different conditions. All experiments were done with same Asp. flow rate (20 μL/s), Disp. flow rate (100 μL/s), penetration depth (27 mm), delay between aspirations (10 sec.), aspiration volume (500 μL), waiting time before sampling (1 min.). Each experiment conducted twice (Trial 1 and Trial 2) for reproducibility

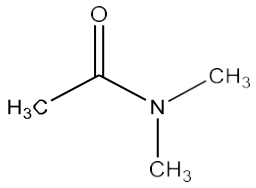
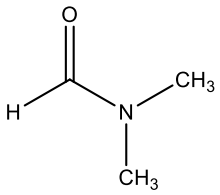
variable		Exp. 7	Exp. 8	Exp. 9	Exp. 10
Composition of solution					
SS (μL): 0.25 M methoxy phenylboronic acid in DMA		50	50	40	30
DCM (μL)		950	950	960	970
Diol resin (equiv.)		12	12	**15**	**20**
# of aspiration-dispensing		15	**30**	15	15
Phenylboronic acid removal	Trial 1	71%	75%	81%	85%
	Trial 2	73%	75%	82%	86%

2.2.2. Results of Experiments with DMF (Dimethylformamide) as the Solvent for standard solution

Recognizing that further increasing the scavenger equivalents might not lead to the desired extraction efficacy, attention was turned to the solvent used for the standard solution (standard solution: 0.25M phenylboronic acid, which is the concentration of remaining unreacted Phenyl boronic acid in the Heck reaction mixture and before any scavenging process).

The hypothesis was that using a less polar solvent such as DMF (dimethylformamide) might result in weaker interactions between it and the phenylboronic acid (**Table 2.5**). Consequently, phenylboronic acid would be more available to react with the scavenger, potentially increasing scavenging efficiency.

Table 2-5 comparison of structure and properties of DMA (Dimethylacetamide) and DMF (Dimethylformamide)

Structure		
Name	Dimethylacetamide (DMA)	Dimethylformamide (DMF)
Melting point (C)	-20	-60
Boiling point (C)	166	152
Dielectric constant	37.8	36.7

Thus, in Experiment 11, a switch to using DMF as the solvent for the standard solution was made, while keeping the scavenger equivalents and the number of aspiration-dispensing cycles the same as in Experiment 7. The result was remarkable, with the scavenger achieving an efficiency of 95%. This significant improvement (compared to 73% in Experiment 7) indicated that even a small difference in the polarity of the solvent had a substantial effect on scavenger performance.

For Experiment 12, the scavenger equivalents were reduced to six, while maintaining fifteen aspiration- dispensing cycles. This change resulted in a reduction in efficiency to 88%. However, increasing the number of aspiration-dispensing cycles to 30 while maintaining 6 equiv. of scavenger in Experiment 13 led to an improvement in efficiency, reaching 91%.

Considering the conditions of Experiment 11 (12 equiv. of scavenger, 15 aspiration-dispensing cycles), Experiment 14 was a reduction in scavenger equivalents to eight while increasing the number of aspiration-dispensing cycles to twenty. Interestingly, despite the change in scavenger equivalents and aspiration-dispensing cycles, the efficiency remained consistent with Experiment 11 at 95%. Therefore, both Experiment 11 and Experiment 14 can be considered optimum conditions for scavenger performance.

A summary of the parameters and results of Experiment 11-14 provided in **Table 2.6**.

Table 2-6 Results of scavenger efficiency in Robotic experiments (Exp. 11-14) using different conditions. All experiments were done with same Asp. flow rate (20 μL/s), Disp. flow rate (100 μL/s), penetration depth (27 mm), delay between aspirations (10 sec.), aspiration volume (500 μL), waiting time before sampling (1 min.). Each experiment conducted twice (Trial 1 and Trial 2) for reproducibility assessment.

variable		Exp. 11	Exp. 12	Exp. 13	Exp. 14
Composition of solution					
SS (μL): 0.25 M methoxy phenylboronic acid in DMF		50	50	50	50
DCM (μL)		950	950	950	950
Diol resin (equiv.)		**12**	6	6	**8**
# of aspiration-dispensing		**15**	15	**30**	**20**
Phenylboronic acid removal	Trial 1	**95%**	**88%**	**91%**	**93%**
	Trial 2	**95%**	**87%**	**91%**	**95%**

2.2.3. Effect of Different Phenylboronic Acid Concentrations

In the previous experiments, it was assumed that one equiv. of phenylboronic acid would react completely with butyl acrylate, leaving only one equiv. of phenylboronic acid in the reaction mixture. To assess how the concentration of remaining phenylboronic acid affects scavenger performance, the optimal condition from the previous experiment (Experiment 14) was repeated using varying concentrations of phenyl boronic acid in the reaction solvent (DMF). **Table 2.7** presents the scavenger efficiency for different conversion levels of the oxidative Heck reaction, 0%, 50%, 75%, and 100% conversion to Heck product and by-products, which corresponded to 0.5 M, 0.375 M, 0.3125 M, and 0.25M remaining phenylboronic acid, respectively.

Table 2-7 Result of scavenger efficiency in different concentration of phenyl boronic acid in the reaction mixture. All experiments were done with same Asp. flow rate (20 μL/s), Disp. flow rate (100 μL/s), penetration depth (27 mm), delay between aspirations (10 sec.), aspiration volume (500 μL), waiting time before sampling (1 min.), and 20 times aspiration/dispensing cycles. Each experiment conducted twice (try 1 and try 2) for reproducibility assessment.

Entry	Reaction conversion	Remaining Boronic acid (equiv.)	remaining Boronic acid (M)	8 equiv. Diol resin (mg)	Boronic acid removal
1	0%	2	0.5	175	**98%** (1st try) **97%** (2nd try)
2	50%	1.5	0.375	131	**96%** (1st try) **96%** (2nd try)
3	75%	1.25	0.3125	109	**97%** (1st try) **97%** (2nd try)
4	100%	1	0.25	87	**93%** (1st try) **95%** (2nd try)
5	100% + by-product 2	0.75	0.1875	65	**95%** (1st try) **96%** (2nd try)

Based on these results, it is evident that, regardless of the initial concentration of phenylboronic acid in the original reaction mixture, the scavenger, under the optimum conditions, is capable of removing ≥ 95% of the phenyl boronic acid. This outcome highlights the robust and efficient performance of the scavenger, ensuring effective removal of the target compound, even in scenarios with varying phenyl boronic acid concentrations.

3. Conclusions and Future Outlook

In this study, we have successfully developed an automated sample preparation method tailored for gas chromatography, a widely used and popular technique in analytical chemistry.

A widely recognized reaction, the base-free, oxidative Heck reaction between 4-Methoxyphenylboronic acid and n-Butyl acrylate and palladium (II) acetate as catalyst, was used as the model reaction. Method development was based on removing 4- Methoxy phenylboronic acid from the reaction mixture prior to GC injection, a task crucial for clean injection to prevent column degradation. Different parameters were considered to be optimized, such as the amount (number of equiv.) of scavenger with respect to 4- methoxy phenylboronic acid, the number of mixing cycles (aspiration-dispensing), the delay time between mixing cycles, the choice of solvent, and the concentration of 4-Methoxyphenylboronic acid in the solution. Our findings demonstrated that eight equivalents of Diol resin scavenger with respect to 4- methoxy phenylboronic acid, regardless of concentration (within the range of 0.5 M to 0.18 M, in a mixture of 50 μL DMA and 950 μL DCM (1 mL total), can successfully remove more than 95% of boronic acid form the mixture. This optimal condition corresponded to 20 aspiration-dispensing cycles, 10 sec. time interval between mixing cycles, and flow rates of 20 μL per sec. for aspiration and flowrate of 100 μL per sec. for dispensing.

While this method represents an innovative and simple automated sample preparation for gas chromatography, it is important to acknowledge its current limitation - the need for manually prefilled vials with scavengers. However, new markets for analytical instruments supply new versions of liquid sampling equipment equipped with weighing features, allowing for automated measurements of precise material quantities and automatic filling of vials with solid powders.

This study is a step forward in replacing packed-columns or cartridges with suspended powdered forms of scavengers simplifying the workflow and enhancing efficiency.

Future study on this platform should involve assessing the reusability of scavenger through a catch-and-release mechanism aiming to find an appropriate solvent to free up the boronic acid from the scavenger, which can introduce an element of enhanced sustainability into the process.

Another step toward the method's development involves expanding its scope to encompass the removal of not only methoxy phenylboronic acid but also the Pd catalyst and any of its byproducts. The latter, another compound within the oxidative Heck reaction, must be eliminated from the injectable sample prior to GC analysis, as it can induce column degradation.

4. Experimental

4.1. Chemicals and Reagents

4-methoxyphenylboronicacid (>95.0%), n-butyl acrylate (>99%), p-benzoquinone (>98%), neocuproine (1,10-2,9-dimethyl phenanthroline), palladium (II)acetate (98%), N,N-Dimethylacetamide (DMA), Dimethylformamide (DMF) were purchased from Sigma Aldrich Company and were used without further purification. Acetone, hexanes, and ethyl acetate were purchased in bulk from Fisher Scientific and were used without further purification. Dichloromethane (DCM) and diethyl ether (ether) were purchased from Millipore Sigma Ltd. Deuterated chloroform (Chloroform-D without TMS) and deuterated dimethyl sulfoxide (dmso-d6) were purchased from ACP Chemicals Inc. Company. SilliaBound Diol resin (1.14 mmol/g) in bulk powdered form supplied by Silicycle Inc.

4.2. Equipment

Analytical thin layer chromatography (TLC) was performed on 200 µm thick silica gel (Silicycle); spots were visualized with UV light (254 nm). Column chromatography purifications were carried out using Biotage Isolera instruments on Santai iLOK-SL silica gel (40 - 63 µm, 60 Å) columns. Microwave-assisted heating was performed in tightly capped vials (Biotage or Chemglass) in a Biotage Initiator microwave oven. The robotic liquid handling system RTC (robotic tool change) used for automated experiments was a CTC Inc. PALsystem liquid handler. Manual experiments were done in 3mL glass vials, automatic experiments were done in 1.5 mL standard GC vials. Solution NMR spectra were recorded on a Bruker AVANCE III 500 MHz spectrometer. 1H NMR spectra were internally referenced to TMS. Personal computer was used for controlling the parameters of automatic scavenger experiments.

4.3. Batch procedure for Oxidative Heck Reaction between Butyl acrylate and Boronic Acid

In a small glass vial, palladium acetate (2.25 mg, 0.01 mmol) and dimethyl phenanthroline (2.5 mg, 0.012 mmol) in 1 mL of DMA was stirred for 30 min. to form the active catalyst (**Figure 4.1.a**). In a 5 mL microwave transparent process vial, 4-methoxyphenylboronic acid (152 mg, 1 mmol), n-butyl acrylate (72 μL, 0.5 mmol) and p-benzoquinone (54 mg, 0.5 mmol) were dissolved in 1 mL of DMA (**Figure 4.1.b**). The content from the vial was added to the process vial (**Figure 3.1.c**), which was further capped and exposed to microwave heating (100 °C) for 10 min.

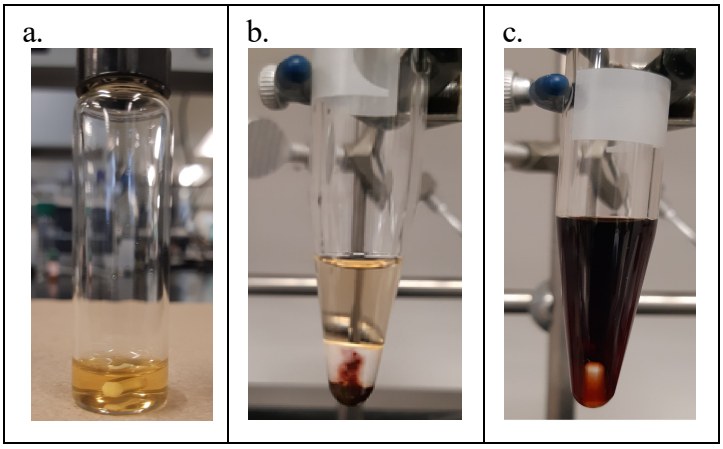


Figure 4.1 visual appearance of the manual oxidative Heck reaction between butyl acrylate and 4-methoxyphenyl boronic acid at various stages of the process. **a**. Palladium acetate and dimethyl phenanthroline in 1 mL DMA after 30 min. stirring. **b**. 4-methoxyphenylboronic acid, n-Butyl acrylate, and p-Benzoquinone in 1 mL DMA. **c**. Reaction mixture after mixing all reagents.

After microwave heating, the reaction vessel was thereafter cooled to room temperature and the crude reaction mixture was diluted with ethyl acetate (EtOAc, 50mL) and extracted with water (3 x 50 mL) and the pooled organic layer washed with brine and dried over anhydrous $MgSO_4$. Following filtration, the solvent was evaporated under reduced pressure using rotavap and 241 mg yellow, oily residue obtained. The residue was purified by column chromatography using 20% ether in hexane as eluting solvent. During the column chromatography process, three distinct components were detected. The eluting fractions were collected in separate test

tubes to isolate and characterize each component individually. After removing the solvent of each fraction, the isolated mass of each fraction was 6.7 mg, 87.4 mg, and 14.1 mg respectively.

To gain further insights into the composition and structure of each fraction, analysis by gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy were performed (**Figure 4.2**). The yield of the desired product was determined to be 74.7%.

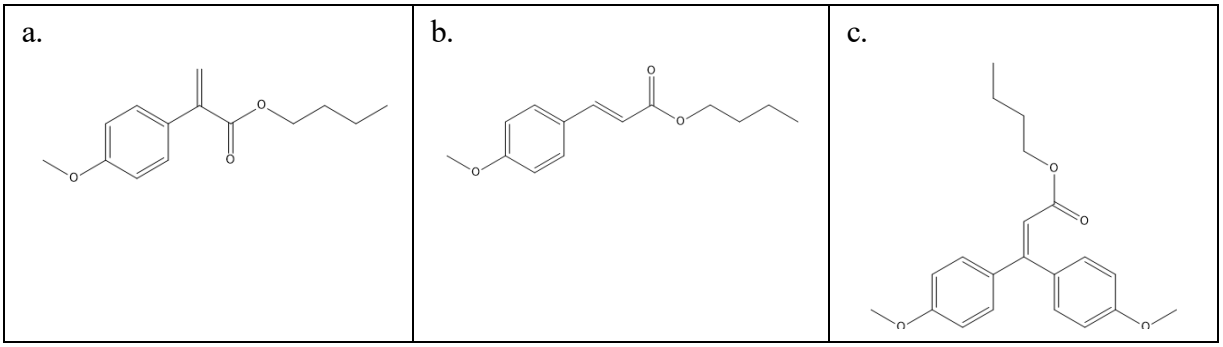


Figure 4.2 Structure of three purified compounds obtained after column chromatography, GC-MS and NMR spectroscopy analysis. a. structure of the by-product 1 (MW= 234.1 g/mol). b. structure of desired product (MW= 234.1 g/mol). c. Structure of the by-product 2 (MW= 340.1 g/mol).

4.4. General scavenging procedure for 4-methoxy phenylboronic acid removal

The procedure for utilizing the SiliaBondDiol resin as a solid scavenger involved several steps to ensure efficient scavenging and isolation of phenylboronic acid.

- Firstly, a mixture, containing phenylboronic acid in DMA (the reaction solvent), was transferred to a vial that had been pre-filled with the SiliaBondDiol resin.
- Subsequently, the mixture and the SiliaBondDiol resin were mixed for a specific duration and intensity. This mixing step was crucial to ensure thorough contact between the resin and the phenylboronic acid, facilitating the binding and removal of phenylboronic acid by the resin. The mixing process was optimized to enhance the efficiency of scavenging, allowing the resin's diol groups to interact with the

phenylboronic acid and form stable complexes.

- After the completion of the mixing step, the vial was left undisturbed, enabling the precipitating of the solid powder scavenger. The SiliaBondDiol resin, along with the scavenged phenylboronic acid, settled at the bottom of the vial.
- Subsequently, the upper portion of the liquid, which contained leftover dissolved phenylboronic acid, was carefully transferred to the next vial for further analysis.

It is important to note that in the first Step of the procedure, only phenylboronic acid in DMA (the reaction solvent) was utilized, rather than all the components present in the oxidative Heck reaction. However, the phenylboronic acid was used at the same concentration as in the original reaction mixture (0.25 M). This modification was implemented to accurately investigate the effect of the SiliaBondDiol resin on phenylboronic acid alone.

4.5. Setup of the Automated System and the Role of Robot Arm in Sample Preparation Process

In this study, an automated system was employed for sample preparation. The core component of the automated system was a robot arm (PAL3 CTC robot for sample preparation) equipped with a 1 mL micro-syringe (**Figure 4.3**).

Figure 4.3 PAL3 RTC robot for sample preparation

The vials used in the automated system were arranged in two rows on a vial tray. The first row of vials included Vial 1, containing the prepared solution of phenylboronic acid in DMA (with 1 equiv. of Toluene as internal standard), Vial 2 contained DCM, Vial 3 contained a specific amount of the diol resin scavenger, and Vial 4 was an empty vial intended to hold the final mixture after the scavenging process (**Figure 4.4**).

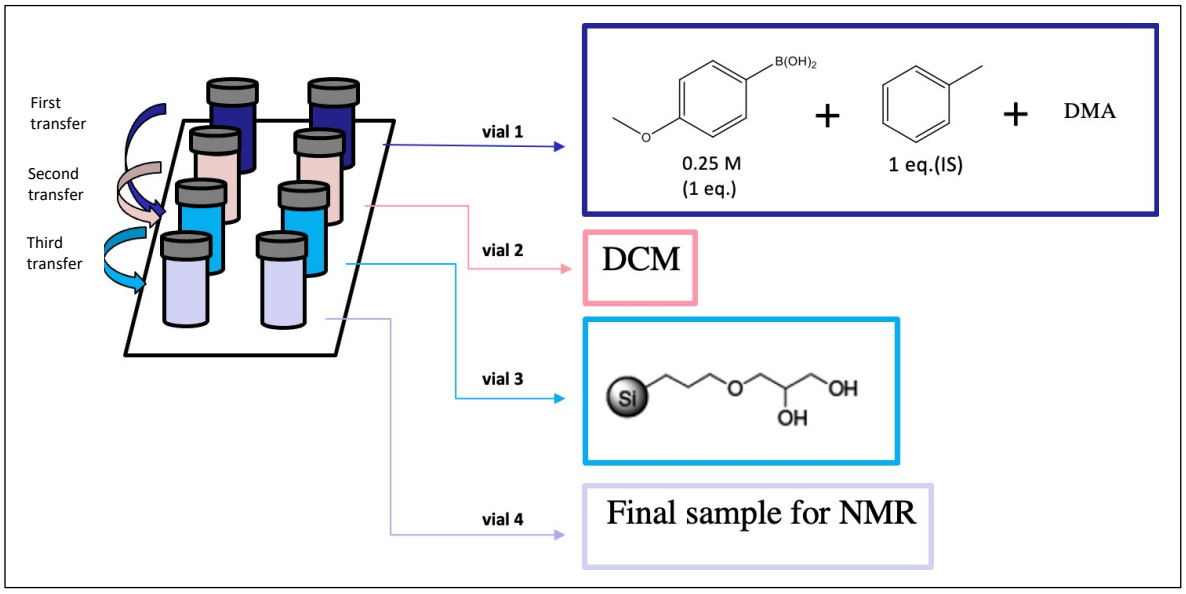


Figure 4.4 Arrangement of vials for automatic scavenging procedure

- Transferring Mixture Containing Phenylboronic Acid from Vial 1 to Scavenger-Contained Vial 3 (First Transfer)

The first step of the automated sample preparation process involves transferring the mixture containing phenylboronic acid from Vial 1 to Vial 3, which contains the diol resin scavenger. The robot arm maneuvers the syringe to the top of vial 1 and penetrates the vial through the septum cap. It aspirates 50 μL of the mixture and then moves the syringe out of Vial 1. Subsequently, the robot arm positions the syringe above Vial 3, penetrates the septum cap, and dispenses all 50 μL of the mixture into Vial 3.

- Dilution with DCM (Second transfer)

In the second step, the robot arm transferers 950 μL of DCM from Vial 2 to the mixture

of phenylboronic acid and the scavenger in Vial 3. It aspirates the DCM from Vial 2 and then dispenses it all into vial 3.

- Mixing the Mixture in Vial 3

To achieve efficient scavenging and thorough mixing of the reaction components, the robot arm keeps the syringe's needle in Vial 3 while aspirating and dispensing a specific amount (500 μL) of the top portion of the mixture for a designated duration. During this mixing step, the syringe is programmed to remain motionless for a predefined waiting time after each dispensing, allowing solid particles to settle down at the bottom of Vial 3. This crucial waiting time ensures that the scavenger effectively interacts with and scavenges phenylboronic acid from the mixture. After the waiting time elapses, the syringe aspirates 500 μL of the top transparent portion of the mixture, and the robot arm moves the syringe out of Vial 3.

- Transferring the Top Transparent Portion of the Mixture to Vial 4 (Third Transfer)

The final step involves transferring the top transparent portion of the mixture in vial 3, to Vial 4 for further analysis. The robot arm moves the syringe to the top of Vial 4, penetrates the septum cap, and then dispenses all 500 μL of the mixture into Vial 4.

- Repeating the Experiment

For ensuring the reliability and reproducibility of the results, the second row of vials on the vial tray was identical to the first row. This repetition allows for conducting the experiment under the same conditions, verifying the consistency of the scavenging process performed by the SiliaBondDiol resin in removing phenylboronic acid. By conducting the experiment in duplicate, any potential sources of variation or experimental errors could be identified and addressed, enhancing the overall confidence in the study's outcomes.

- Comparison of NMR Spectroscopy Results for Vial 1 and Vial 4

With the automated sample preparation process complete, Vial 4 now contains the reaction mixture after efficient scavenging of phenylboronic acid by the diol resin. Vial 1, on the other hand, contains the initial mixture with phenylboronic acid before scavenging. To assess the efficiency of phenylboronic acid removal, the ^{1}HNMR spectroscopic results of both Vial 1 and Vial 4 is compared. By comparing the HNMR spectra of the solution before and after scavenging, we can determine the extent of phenylboronic acid removal achieved by the scavenger process.

4.6. Considerations for the Automated Scavenging Process

The success of the scavenging process relies on various parameters that influence the mixing intensity and effectiveness. To ensure optimal mixing and, consequently, effective scavenging, a custom-made program was developed to control the robot and syringe using specific parameters. The details of each parameter and their importance in the sample preparation process are discussed below.

4.6.1. Parameters that Influence the Mixing Efficiency

- **Aspiration Flow Rate:**

The aspiration flow rate dictates how quickly the syringe takes the liquid from the reaction mixture (**Figure 4.5.a**). This parameter needs to be set to a slow enough rate to prevent disturbing the mixture, which could lead to the movement of solid scavenger particles, potentially reaching the syringe needle and causing clogging issues. A controlled and gentle aspiration flow rate ensures the stability of the mixture while allowing the syringe to take a representative sample for further processing.

- **Dispense Flow Rate:**

The dispense flow rate is the rate at which the syringe empties and delivers back the liquid it previously aspirated (**Figure 4.5.b**). This flow rate plays a crucial role in achieving an efficient and intense mixing of the reaction components. Since the scavenger is in suspended solid particles, it is essential to ensure a sufficiently fast dispense flow rate to agitate the mixture effectively. The intense mixing helps move the solid particles from the bottom of the vial, enabling thorough interaction with the reaction components.

- **Penetration Depth:**

The penetration depth refers to the distance from the cap of the vial to which the tip of the syringe needle descends during the aspiration and dispense steps. The appropriate penetration depth is critical to optimize the mixing efficiency. If the penetration depth is too shallow, the needle might remain near the surface of the mixture, leading to minimal liquid aspiration and low mixing intensity during the dispense step. On the other hand, if the penetration depth is too deep, the needle might come close to the settled solid scavenger particles at the vial's bottom. This could result in some solid particles being inadvertently drawn into the needle, leading to needle clogging. Thus, finding an optimum penetration depth is crucial to strike a balance between effective mixing and preventing clogging.

Based on the experimental setup and vial dimensions, the total height of the vial was determined to be 32 mm, with the solid scavenger occupying the lower 3 mm from the bottom. Considering that the total volume of the mixture was approximately 1 mL and that about 0.5 mL of the top liquid was sufficient for each aspiration/dispense step to achieve intense mixing, a safe penetration depth of around 27 mm was identified as the ideal position for the needle to remain during the process (**Figure 4.5.c**).

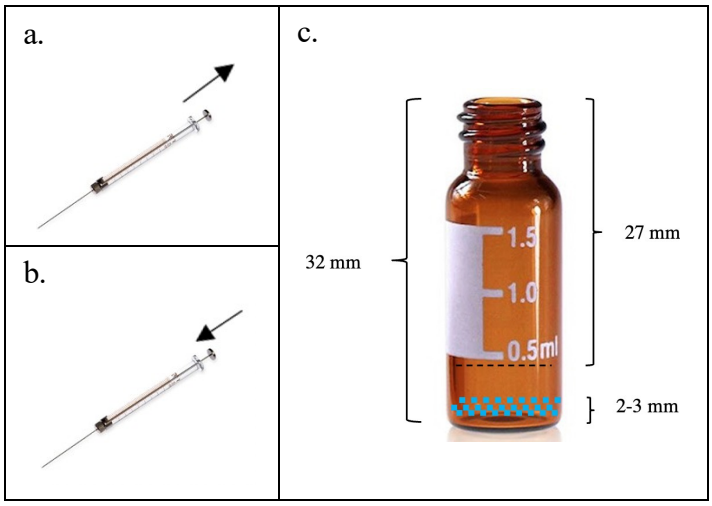


Figure 4.5. a. aspiration. **b.** dispensing. **c.** dimensions of the vial. Optimum penetration depth was considered 27 mm.

- **Number of Aspiration/Dispensing:**

The number of aspiration/dispensing cycles translates to the reaction time between phenylboronic acid and the scavenger. Increasing the number of aspiration/dispensing cycles allows for longer interactions between the scavenger and the phenylboronic acid in the reaction mixture, potentially improving scavenging efficiency.

- **Aspiration Volume:**

The aspiration volume is the amount of liquid that the syringe takes during each aspiration/dispensing trial. This volume must not exceed the syringe's capacity, which in this case is 1 mL. Furthermore, the penetration depth determines the amount of liquid available above the tip of the needle for aspiration. Considering these factors, the aspiration volume should be set equal to or less than 0.5 mL (500 μL) to ensure successful mixing without exceeding the syringe's capacity or encountering clogging issues.

- **Delay Time between Each Dispensing and Next Aspiration:**

The delay time is the interval between each dispense step and the subsequent aspiration. This delay allows sufficient time for the solid scavenger particles to settle down at the bottom of the vial, ensuring a consistent starting point for each aspiration. Although theoretically, this delay time could be quite long, in practice, it is optimized to balance automation efficiency with sufficient settling time. By reducing the delay time and the number of aspiration/dispensing cycles, the sample preparation time can be minimized, a crucial aspect of automation.

4.6.2. Importance of Optimizing Parameters

The precise control of these mixing parameters is of utmost importance in achieving efficient scavenging. A well-optimized mixing process ensures that the scavenger particles disperse evenly throughout the reaction mixture, maximizing the chances of interaction with the phenylboronic acid. The optimized mixing process also ensures consistency in sample preparation across multiple experiments, minimizing experimental variations and enhancing the overall reliability of the results. With automation and precise control over the mixing parameters, we can confidently replicate the sample preparation process, further increasing the reproducibility of the study.

4.6.3. NMR Analysis and Internal Standard

To enable accurate comparison between the NMR spectra of the mixture before and after scavenging process, an internal standard as a reference peak was introduced to the samples. It is crucial to select an internal standard that does not overlap with the peaks of interest, including phenylboronic acid and the solvents (DCM, DMA, and DMF).

For this purpose, toluene was chosen as the internal standard. Toluene was added to the standard solution (0.25 M 4-methoxyphenyl boronic acid) at the beginning of the process before the scavenging process started.

4.6.4. Calculating the Efficiency of Scavenging

In the ^{1}HNMR spectrum of standard solution, the peak corresponding to 4-methoxy phenylboronic acid can be observed, along with the peaks of the solvents and other reaction components. Similarly, in the ^{1}HNMR spectrum of the processed mixture, the 4-methoxy phenylboronic acid peaks will ideally be diminished or absent, indicating successful removal of the analyte by the scavenger.

To determine the efficiency of 4-methoxy phenylboronic acid removal by the scavenging process, the molar ratio of 4-methoxy phenylboronic acid to toluene is calculated for both processed and unprocessed mixtures. By comparing these molar ratios, we can quantitatively assess the scavenger's performance. A higher reduction in the molar ratio signifies a more efficient scavenging process, indicating successful removal of 4-methoxyphenylboronic acid from the reaction mixture.

Example calculation of scavenger efficiency in Experiment 1 is provided below (**Figure 4.6** and **Figure 4.7**):

Ratio of 4-methoxyphenylboronic acid to toluene in the is calculated based on two reference peaks; 1- the doublet at 6.90 ppm which corresponds to two hydrogens on phenyl ring in 4-methoxyphenylboronic acid and 2- the multiplet at 7.25 ppm which corresponds to two hydrogens (meta positions) on phenyl ring in toluene.

The ratio of 4-methoxyphenylboronic acid to toluene in the solution before scavenging process form **Figure 4.6** (R1):

R1 = 2.00/1.50 = 1.33

The ratio of 4-methoxyphenylboronic acid to toluene in the solution after scavenging process with one equiv. (molar ratio) of diol scavenger with respect to 4-methoxyphenylboronic acid form **Figure 4.7** (R2):

R2 = 2.00/2.31 = 0.86

Removal efficiency of one equiv. diol scavenger (R_{eff}):

R_{eff} = [(R1 – R2)/R1] x 100 = [(1.33 – 0.86)/1.33] x 100 = 36%

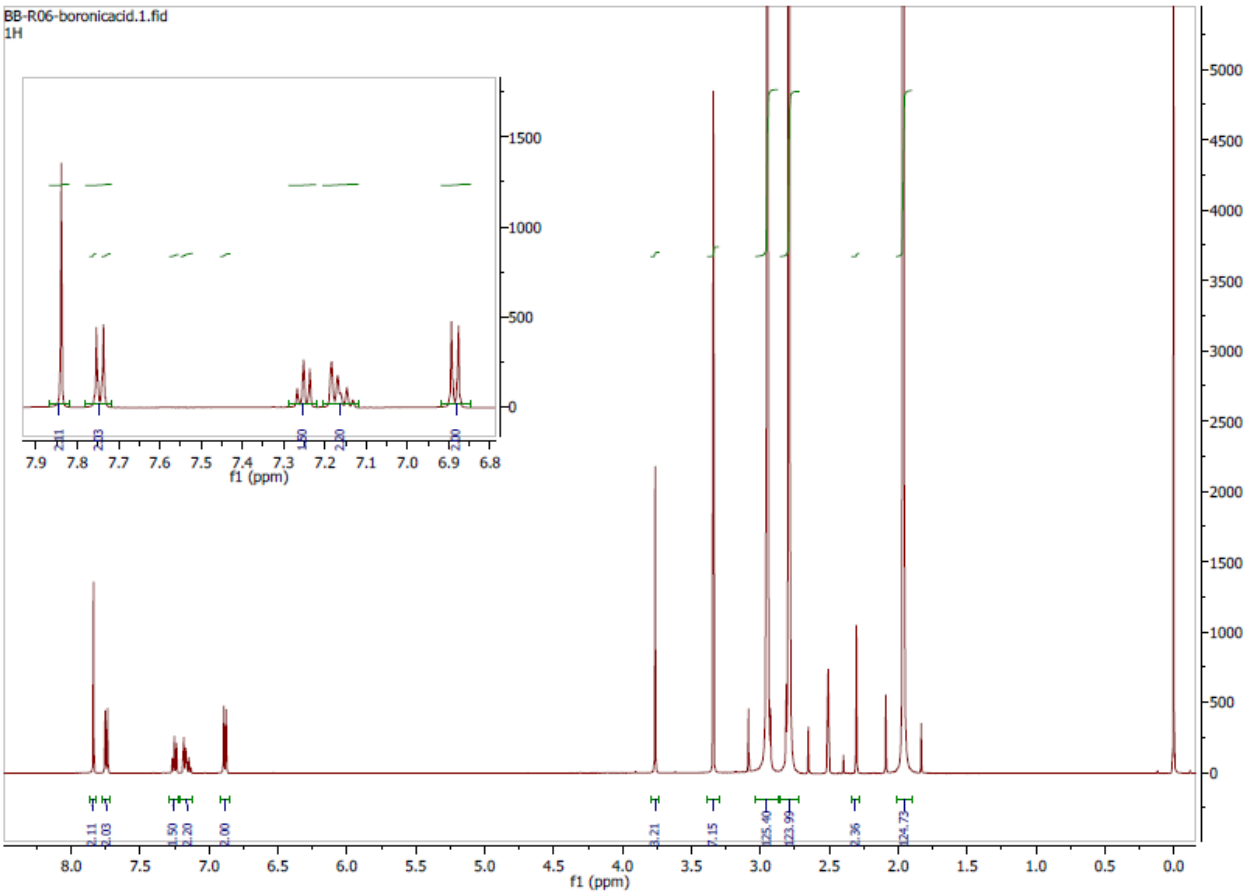


Figure 4.6. [1]HNMR spectrum of standard solution before scavenging process. Solution contains 0.25 M 4-methoxyphenylboronic acid and Toluene (internal standard) in DMA.

Table 4-1. ^{1}H NMR chemical shifts of toluene, DMA, 4-methoxyphenylboronic acid in DMSO-d6.

Compound	Proton	Multiplicity	Chemical Shift (ppm)
Toluene	CH_3	singlet	2.30
	CH (o/ p)	multiplet	7.18
	CH (m)	multiplet	7.25
DMA	CH_3CO	singlet	1.96
	NCH_3	singlet	2.78
	NCH_3	singlet	2.94
4-methoxyphenylboronic acid	OCH_3	singlet	3.78
	CH (o)	dublet	6.90
	CH (m)	dublet	7.75
DMSO-d6	CH_3	singlet	2.50

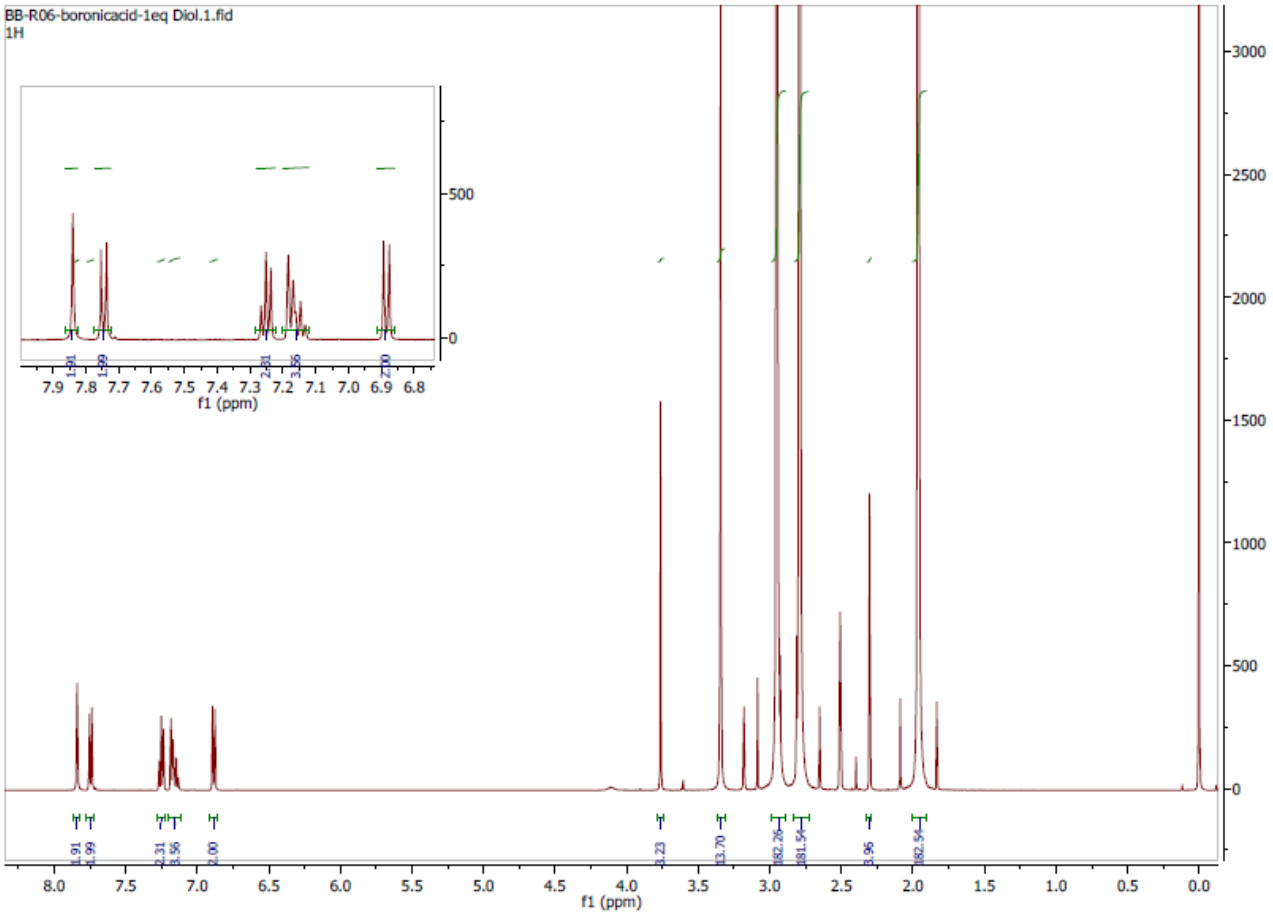


Figure 4.7. ^{1}HNMR spectrum of standard solution after scavenging process with one equiv. (molar ration) of Diol scavenger with respect to 4-Methoxyohenylboronic acid. Solution contains remaining 4-methoxyphenylboronic acid and Toluene (internal standard) in DMA.

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[55] "real-world samples" refer to actual samples that are encountered in practical, everyday situations. These samples are not artificially created in a laboratory but are representative of the diverse and complex substances found in various environments.