High-Throughput Experimentation as an accessible technology for academic organic chemists in Europe and beyond.

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Abstract: For years now, High-Throughput Experimentation (HTE) have been applied to organic chemistry for reaction optimization and reaction discovery as a powerful tool for time and cost reduction. If this technology has been first developed by and for industry, and used as a routine method today, some academic researchers, including in Europe, are still challenging the accessibility of HTE as a general and daily used technology. One of the reasons is probably the expensive cost of such facilities development, which generally involves automation with robots, dedicated research teams, and expensive analytical instrumentation. This paper aims at bringing to light the accessibility of HTE with a minimum of instrumentation and cost, in order to help organic chemists to accelerate the discovery and optimization of new synthetic methodology, leading them to reduce their costs and empower their innovative research.

Introduction

For a few decades now, High-Throughput Experimentation (HTE) has revolutionized the way researchers are designing and performing their experiments in pharmaceutical companies. HTE is described as a miniaturization and parallelization of reaction conditions, thus allowing a large number of experiments to be performed with minimal material consumption and reducing significantly the amount of time required for reaction discovery and synthetic development.[1] Therefore, this technique has emerged as a powerful tool to speed up both processes and has made perfect sense to answer some of the current problems in pharmaceutical industry. In fact, these industries are continuously working on reducing the costs of active pharmaceutical ingredients (APIs) development, and reaction optimization represent a time- and resource-consuming step in drug development. Thus, several industry laboratories (Merck, GSK, Janssen, Pfizer, BMS, etc.)[2] has deployed resources and specific research teams to establish their HTE facilities. These facilities are generally equipped with robotic equipment to setup the experiments and with state-of-the-art analytical instruments for fast analysis. Hence, large arrays of reaction conditions can be evaluated in a reasonable time frame. HTE has been strongly seized and empowered by some of these companies, leading to robust and consistent techniques for a wide variety of chemical reactions commonly used by an organic chemist. Driven by the advances in technology, HTE platforms developed for synthetic organic chemistry have evolved rapidly during the last decade, from standard screenings protocols performed at micromole scale in a 96-well plate format, to HTE campaigns being currently performed at nanomole scale in 1536-well microtiter plates. To execute reactions in such level of miniaturization, high-precision nanoliter liquid handling robotics are needed and high throughput analytical methods to analyze a large number of samples in a reasonable time frame must be in place.[3] Merck researchers recently proved the efficiency of such nanoscale approaches to map the chemical space of several reactions commonly used in medicinal chemistry.[4] Noteworthy, the combination of machine learning (ML) based algorithms with HTE has emerged as a powerful tool to expedite the exploration of chemical space. In this context, HTE have been used to quickly generate high-quality datasets to feed ML algorithms in a cost-effective manner and to quickly find suitable initial reactivity points to further optimize them by using ML approaches.[5] Recently, the development of new approaches based on rapid automated experimentation to study both discrete and continuous variables that would produce more rapidly scalable results are gaining interest among the scientific community.[6] Today, HTE is considered for most of the pharmaceutical companies as a routine process. At the contrary, this challenge has been only recently tackled by the academic research and has grown timidly. Even though the pressure in academia is not the exact same one as it is in pharmaceutical industry, publishing innovative research with a minimum of time and resources is of huge interest for academic research groups, particularly in a context of reduction of resources, and in highly competitive research topics. One of the first academic HTE facilities was established at the University of Pennsylvania with the help of Merck in 2009. Since then, a number of HTE facilities have been created in USA, leading to a large range of HTE-related publications (see Figure 1).[7] At present, several research groups in USA are using this technique routinely, thanks to a privileged access to one of these facilities.

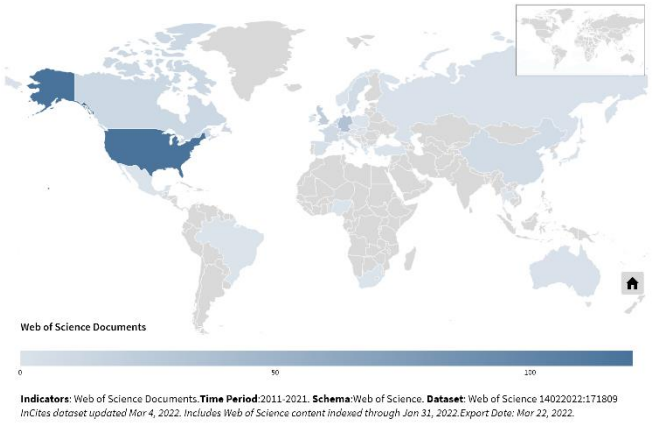


Figure 1. Worldwide state of the art of HTE (2001-2021), from Web of Science by Clarivate.

However, this technology is not yet accessible to the main part of academic research groups, as in Europe for instance, with a very limited number of HTE centers developed, and a difficult access of external researchers to these facilities.[8] One of the most important reasons is the high cost of deploying such facilities. In fact, setting up a HTE facility from scratch can represent a significant expense, from gloveboxes to liquid and solid dispensing robots, including analytical instrumentation and employment of dedicated people. With time and experiences, we learned how to identify key parameters and the instrumentation needed for basic and routine HTE experiments in academic settings. In fact, some instrumentation particularly appreciated in industry, as fully automated workstations, might not be ideal for academic research. As far as we know, current commercially available automated HTE platforms for reaction optimization and reaction discovery are quite expensive and not so versatile. By this we mean that the experimental protocols required for setting up HTE screenings must typically be tailored for each particular type of reaction thus requiring time and effort to configure all the parameters in order to have a robust and reliable HTE platform. Moreover, as each research project is drastically different in multidisciplinary academic centers, and the flow of experiments is not as substantial as it can be in pharmaceutical companies, it could lead to unprofitable maintenance costs.[9]

With this discussion, we expect to take advantage of our respective experiences in academic HTE centers to highlight the essential instrumentation required for efficient, flexible and sustainable HTE in the realm of organic chemistry, and to demonstrate the accessibility of this technology, with in mind helping a wide range of organic chemists to empower their innovative research, by accelerating their optimization reaction steps.

Discussion

Classical reaction discovery and optimization generally starts with scientific intuition based on mechanistic rationale and literature precedents. The set of rationally selected variables are typically interrogated following a sequential optimization, leading to the optimization of one variable at a time. Even though this approach can be successful, it can also be a time- and resource-consuming process, and it might end up without the guarantee of finding out the perfect combination of parameters (see Figure 2).

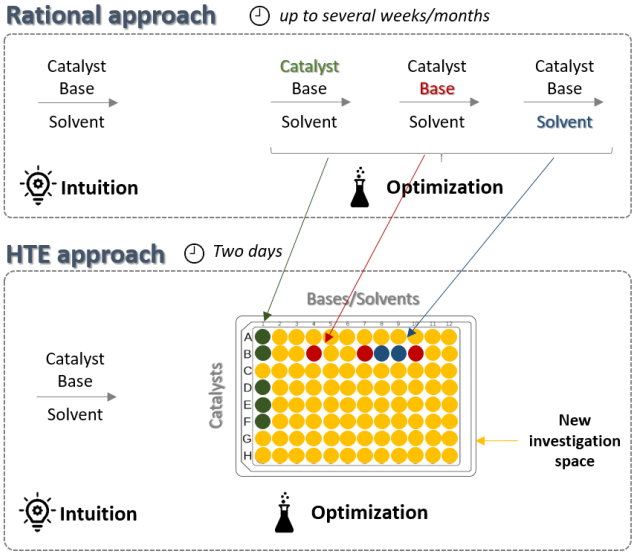


Figure 2. Classical one-variable-at-a-time approach versus multivariate HTE approach.

High-Throughput Experimentation affords solutions to some of these drawbacks. For instance, cross-matching several variables in the first screening campaign allows covering a wider part of the chemical reactivity space within a shorter time frame. Thus, it can rapidly provide a much more detailed knowledge about the chemistry of the reaction to further improve experimental conditions upon. Depending of the outcome of the first screening campaign, the discovery or the optimization process can be followed by a more targeted screening or by traditional batch chemistry. Moreover, the intrinsic miniaturization of reactions in HTE, implies a significant reduction in starting materials and reagents (catalysts, ligands, additives, etc.) consumption, thus making the overall process of discovery and optimization greener and more sustainable if compared with traditional means of experimentation.

However, it is important to have in mind that plate-based HTE techniques are not well suited to precisely interrogate continuous variables such as temperature, pressure, and reaction time, which are extremely important for some reaction optimization processes and in scalability studies. In this sense the combination of HTE (to explore discrete variables: catalyst, ligands, additives, solvents…) and statistical approaches such as DoE (to explore continuous variables: reaction temperature, reaction time, stoichiometry, reaction concentration…) has emerged as a powerful tool for reaction discovery and optimization.[10] Yet it is important to note that with this plate-based HTE approaches not all types of reactions and experimental setups that are commonly used by a synthetic chemist will be realizable. In fact, when working at microscale and in a reduced footprint some experimental procedures might be difficult to perform accurately (e.g. slow addition of reagents, sampling, online analysis).[9] Actually, one of the main challenges surrounding the implementation of HT methods in different types of chemical reactions revolves around the development of suitable experimental technologies and experimental protocols that can provide robust and reliable HTE platforms. This usually requires an earnest effort to develop and test these platforms prior to their use in different research projects. However, once the screening platform and their experimental protocols have been developed, the chemical experimental part of the different related research projects can be highly accelerated.

Moreover, the developed HT platforms and HT protocols can be improved upon based on the experience gained for each research project and used in the future for other members of each research group.

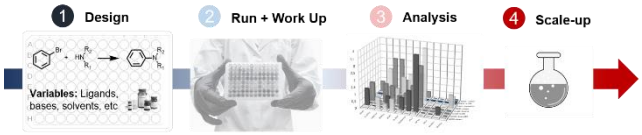


Figure 3. Different steps of a HTE experiment.

In the following paragraphs, we will describe the different steps of a general 96-well HTE experiment and the basic instrumentation needed to perform the screening in academic research laboratories. Some recommendations based on our experience for obtaining consistent results without the aid of automated solid and liquid dosing dispensing robots and problematic points that might arise when setting up and analyze the experiments will be also highlighted. The case example will be designed for a non-air-sensitive reaction, performed in a 96-well reactor containing 1mL glass vials.[11] In case of air-sensitive reaction, the whole protocol can be performed in a glovebox. As a note, the evaluated cost is an average cost based on suppliers and experience.

Design of the experiment. The most important aspect when designing a HTE screening is to change the traditional mindset of exploring single reaction conditions in consecutive experiments. Thus, it is important to avoid thinking in different 96 single reaction conditions, instead one have to select the variables that can affect the process and array these variables in the 96-well plates in order to interrogate them depending on their importance. In this sense, the most important variables will have the largest dimension of the array while minor variables will occupy smaller fractions in the reaction plate. By using this multivariate screening approach, the chemical reactivity space explored will be maximized. Alternatively, it can be useful to perform a single variable screening when a variable emerges as the most important one or when the explored variables do no interact amongst themselves.[12] Yet, the HTE experiment has to be designed based on the experimental feasibility of the whole protocol. For instance, without precise solid/liquid dispensers,[13] the screening of 96 different ligands would imply a hard and tedious work. To solve this drawback, the previous preparation of bespoke libraries of predosed reagents in reaction vials has emerged as a possible solution to facilitate the experimental setup for these single variable screenings.[12a]
Moreover, a major issue raised in the experimental design is how to dose with high integrity all the reagents. When working at microscale, direct weighing of the reagents into the reaction vials is not recommended. Indeed, for reagents that are used in substoichometric quantities direct weighing will not be feasible. The main approach followed in academic microscale HTE is the use of stock solutions (or slurries) and pipettes. The total amount of each reagent needed for the whole plate is calculated and solubilized in a solvent. Thus, the correct amount of each reagent can be precisely dosed into the corresponding reaction vials using single or multichannel pipettes.[14] In the experimental design, the concentration of the stock solutions, chemical compatibilities among the solvents and the reagents dosed, and the order of addition of the reagents must be carefully considered.
Typically, the last reagents added in the setup will be the substrates in the desired reaction solvent. However, highly reactive reagents and volatiles must be added at the end of the setup. In some cases, dosing together some reagents can be important (e.g. catalyst complexation, reagent preparation).
Several pieces of software have been specifically developed to help researchers in the experimental HTE design.[15] Also, Cook *et al*. proposed a simple protocol for stock solution calculations.[16] Yet this step can easily be performed with Excel. Therefore, this step can easily be performed free of charge, based on the scientists' experience and a strategic study of the plate design.

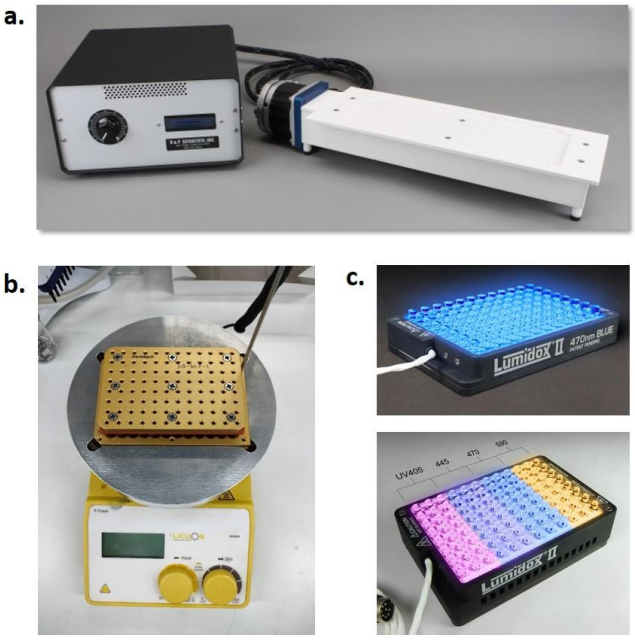
Plate preparation. Without an automated liquid handling dispenser, the stock solutions of the different reagents can be added manually in the corresponding positions of the 96-position reaction plate, with single or multichannel pipettes (Figure 4b). Afterwards, the solvent is usually evaporated leaving the neat reagents (*e.g* metal precursor, ligand, base, additive) dosed in the reaction vial. The same procedure can be used to prepare the bespoke libraries of predosed reagents. As mentioned above, substrates will typically be the last reagents added in the corresponding reaction solvent. Before adding the substrates, stir bars or glass beads will be added into each reaction vial depending on the type of stirring system used. For solvent evaporation, we ideally recommend the use of a centrifuge-evaporator compatible with the SBS plate format (*e.g*. Genevac Evaporator System) (Figure 4d). Alternatively, cheaper evaporation manifolds based on nitrogen or air evaporation are commercially available (Figure 4c).[17] Once all reagents and solvent reactions have been added the 96-well reactor is sealed with a screwdriver and heated to the desired temperature while being stirred.[18] If the reaction is air-sensitive, the plate can be prepared and sealed in a glovebox. The average cost of the plate preparation step will be reaction type-dependent, based on the stability and air-sensibility of reagents. This can vary from 500 (96 well reactor) to 2000$ (96 well reactor + evaporation manifold) of permanent instrumentation and 20$ of consumable items (vials and stir bars). This does not include pipettes and related consumables. In addition, for more permanent evaporation system, a centrifuge-evaporator can be purchased (25k€). As a general note, building HTE devices in house in a mechanical workshop might be an ideal solution to reduce costs.

Figure 4. a) Propylene 96 well LC-plate adaptable in most of analytical instrumentation; b) 96-well reactor plate, c) and d) Evaporation systems.

Figure 5. a) Tumble Stirrer; b) tailor-made aluminum module on the magnetic hotplate stirrer; c) LED 96 well system.

Reaction run. After sealing the reaction vials (Figure 4b), the entire 96-well plate will be submitted to the desired reaction conditions. The safest approach to efficiently stir the 96-well reactors is the use of Tumble Stirrers (figure 5.a).[19] This type of stirring is more compatible with the SBS plate format than the stirring afforded by standard magnetic rotatory stirrers. Alternatively, a magnetic hotplate stirrer can be used to stir and heat homogeneous reactions with appropriate stir bars.[20] In this case, to afford uniform heating across the 96-well plate we recommend adapting a tailor-made aluminum module on the magnetic hotplate stirrer (Figure 5b). However, it is important to note that this type of stirring would not afford uniform stirring across the rectangular SBS format and thus it would not be appropriate for heterogeneous reactions. An alternative consists in the use of an orbital shaker. In this particular case, 3 mm glass beads can be added to the reaction vials to help the stirring process.[21]

In the context of photoredox chemistry, 96-Position LED Arrays with different wavelengths that can be adapted to the bottom part of the 96-well reactors are commercially available (Figure 5.c). However, with these commercially available photoredox HTE platforms the reaction temperature can be difficult to control, especially when working at a high radiant flux. Moreover, the scale-up of hit conditions obtained at microscale can be problematic and further optimization at a larger scale to fine-tune reaction conditions (*e.g*. light intensity, reaction time, and reaction temperature) might be needed. Notably, flow chemistry has emerged as a viable alternative to scale up some photoredox processes.[22] The photon flux passing through the reaction media depends on light intensity and reaction volume and when working at larger scale it might be difficult to match the photon fluxes achieved at microscale. For this reason, we strongly recommend fine-tuning and validating the microscale photoredox HTE platform with a control reaction done with the specific setup used at synthetically useful scales. In order to do so, a control reaction already published displaying similar reactivity can be a good candidate.



To run reactions under pressure of reactive gases, a small number of pressure reactors are commercially available.[23] Alternatively, a basic clamshell pressure reactor could be designed and built in an open-access mechanical workshop (Figure 6b). The mechanical workshops in our respective research centers have been extremely valuable to develop HTE platforms and HTE tools that are not commercially available. For instance, ICIQ has recently developed a HTE platform for reactions that requires a reactant gas and light irradiation (Figure 6a). Although for some specific reaction types in which the scale-up of hit conditions might be problematic and would require further optimization (*e.g*. heterogeneous reactions and photocatalysis); the use of microscale HTE platforms could be extremely useful to quickly explore the chemical reaction space in order to find initial reactivity points to further optimize them at a larger scale. The average cost of the reaction run step will be dependent on the homogeneity of the reaction. This can vary from 0 (room temperature and homogeneous media) to 10k$ (photoredox or heating system, and heterogeneous media) of permanent instrumentation. This does not include pipettes and related consumables. In addition, for high-pressure reaction, homemade pressure reactors can be manufactured to reduce cost.

Work-up. The aim of the workup is to get the samples ready for the analysis. Thus, after the reaction, a minimal and fast workup is done depending on the type of reaction studied. Usually, a solution of a proper internal standard in an organic solvent, compatible with the analytical method of choice, is added with a multichannel pipette in order to compare reaction performance. Alternatively, the internal standard can be added during the reaction setup. If an acid or basic workup is needed, a base or acid compatible with the analytical method of choice can be added to the dilution solution too. For aqueous workups, the internal standard can be added in the extraction solvent. In this later case, we recommend the use of extraction solvents less polar than water; so as to they will remain in the top phase facilitating the withdrawal of the organic aliquot (EtOAc as example).

In case of non-homogeneous solutions, a filtration step might be necessary to remove undesired solid particles. Several 96-well filtering plates with their corresponding vacuum manifolds for simultaneous filtration of the 96 samples are commercially available. Alternatively, the 96-well plate can be centrifuged to expedite solid settling. In this later case, after centrifugation, an aliquot of the homogeneous solution can be easily transferred to a 96-well analysis plate[24] or to glass analysis vials with multi- or single-channel pipettes respectively. One should consider the appropriate final concentration of the analytes and the suitable solvents for preparing the samples depending on the analytical instrumentation used. Then, the analytical 96-well plate is sealed with a commercially available cover mat and submitted to analysis. This step can easily be performed free of charge, by preparing sample vials individually. For sample preparation in a 96 well plate format (if affordable by analytical instrumentation), 20$ of consumable items will be required.

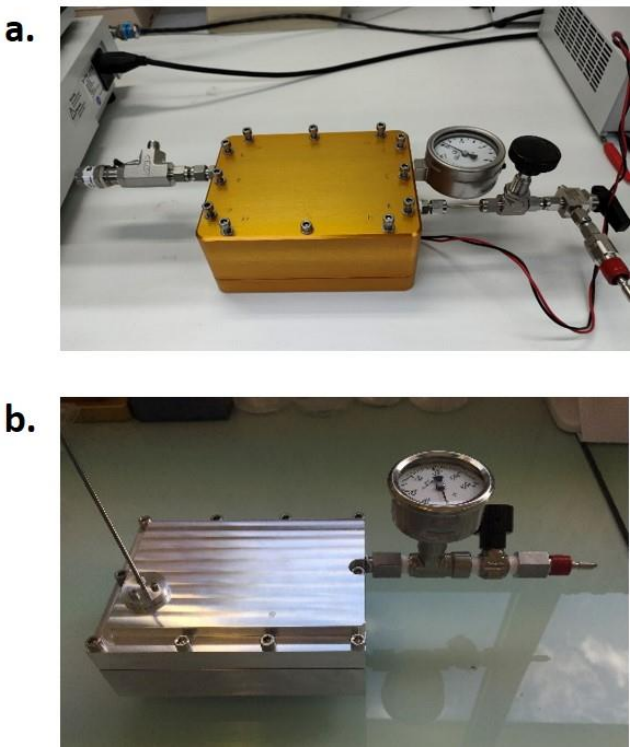


Figure 6. a) Homemade photogas pressure reactor for reactions under pressure and light irradiation at ICIQ; b) homemade reactor for reactions under pressure at CEA.

Analysis. The analytical instrumentation is incontestably the most expensive investment in the development of a basic HTE facility. Yet, nowadays most of the chemistry departments are equipped with analytical instrumentations with plate autosamplers that can be used to analyze the 96-well analysis plates. The analytical part is the centerpiece of HTE thus it is highly important to have a reliable and fast analytical method in hand in order to effectively draw the desired information from the screening campaign in a reasonable time frame. Actually, without a proper analytical method one might end up obtaining non-valuable data or with 96 different problems to solve.

The most widely used analytical techniques to evaluate HTE reaction outcomes in academic synthetic organic chemistry settings are LC (HPLC or UHPLC) coupled with UV/Vis and MS detection for quantification and identification of compounds respectively, SFC for fast determination of enantiomeric enrichment, and GC-MS as a backup option to cover a wider range of analytes and to provide a better chromatographic resolution for samples that are not well resolved by LC analysis.

We strongly recommend developing the analytical methods before performing the screening campaigns. Yet, it is important to note that even if the expected analytes (starting materials, final products, expected byproducts, and internal standard) are well resolved in the initial developed analytical method; other unexpected compounds (ligands, additives, aromatic reaction solvents and unexpected byproducts), present in the crude reactions might coelute with components of interest. In fact, these could complicate data interpretation and it might require reinjecting problematic samples with another analytical method to properly interpret data. The analysis step is free of charge as it considers the analytics present in most chemistry departments. In case of analytics acquisition, the price will vary from 80 to 200k$ (dependent on the instrumentation required and the supplier).

Data Report. The interpretation and reporting of experimental data can be the most time-consuming step in the HTE process without the aid of specific data processing software. In this sense, several companies have rapidly identified the need in the field of High-Throughput Experimentation and have commercialized them at a reasonable price.[25] As a note, it is recommended to scale-up hits conditions observed during the HTE experiment in order to verify the reproducibility of the reaction in a larger scale and to determine or corroborate the yield. The data report step can easily be achieved free of charge by analyzing data on excel sheets.

With the different steps detailed above, even though the human costs are not considered, a basic HTE experiment would be achievable in most of the research labs without a large investment. The total pricing for an experiment will vary from 520$ (500$ permanent instrumentation and 20$ consumable items) and 26k$ for highly sensitive reactions and more permanent investments.

By helping the generalization of this technology, we expect an increase of HTE facilities in chemistry departments in Europe and beyond, allowing the field to evolve and empower even more academic research. The more HTE will be used as a routine in academic research, the more frontier piece fields will develop ergonomic and accessible technologies applied to HTE (analytical methods, related software, etc.).

Conclusion

In this paper, we have highlighted the accessibility of HTE technologies for synthetic development, paving the way to its implementation in other academic centers in a sustainable and cost-effective manner. We do belive that the standardization of High-Throughput Experimentation will expedite experimental work of researchers, thus empowering them to tap their full potential and creativity instead of consuming valuable time in sequential processes of reaction optimization. Despite the accessibility to the technology, it is also important to help the new generation of organic chemists to implement this enabling technology in their research projects. With proper training programs and dissemination of HTE workflows the impact of HTE among the scientific community will be enhanced. It is now the challenge of the HTE practitioners to help scientists to implement this valuable methodology in their research programs.

Acknowledgements

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Keywords: high-throughput experimentation • catalysis • parallel synthesis • micromole scale • miniaturization.

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[14] Pipettes can be calibrated before their use to precisely dose the desired volume, and should be controlled regularly.

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[17] Example of commercially available evaporation systems: EquaVAP 54-Well Evaporator, ref: 23054; supplier: Analytical Sales and Services.

[18] Example of commercially available 96-position reactors: 96-Well Photoredox Block Assembly, ref: 96973; supplier: Analytical Sales and Services; or Reaction block assembly for HTE, parallel synthesis and reaction optimization, ref: VP 416-Ale-96, supplier: V&P Scientific.

[19] Example of commercially available tumble stirrer: Servo Motor powered Rotary Magnetic Tumble Stirrer, high speed and high torque, stirs 2 deep well microplates, ref: VP 710P5X; supplier: V&P Scientific.

[20] More powerful stir bars should be used (*e.g.* .VWR: Ref: 442-0361).

[21] Due to the smaller scale of the reactions, the shaking speed needs to be higher and the amplitude lower than the ones used at a larger scale to obtain comparative results.

[22] a) L. Buglioni; F. Raymenants; A. Slattery; S. D. A. Zondag; T. Noel, *Chem. Rev*. **2022**, *122*, 2752–2906, b) M. González-Esguevillas; D. F. Fernández; J. A. Rincón; M. Barberis; O. de Frutos ; C. Mateos ; S. García-Cerrada ; J. Agejas ; D. W. C. MacMillan, *ACS Cent. Sci*. **2021**, *7* (7), 1126−1134.

[23] Example of commercially available pressure reactor: Pressure Reaction block assembly for HTE, parallel synthesis and reaction optimization, 48 well format, ref: VP 416-ALE-48-HP; supplier: V&P Scientific.

[24] Example of commercially available polypropylene LC-plate: 96-Well Collection Plate with 50uL Tapered-Bottom Wells, ref: 968810; supplier: Analytical Sales and Services.

[25] Examples of softwares dedicated to HTE analysis, see: Virscidian, Katalyst D2D.