Reducing the Bottleneck in Target Synthesis

White Paper

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Executive Summary

Using the Biotage automated workflow, models have shown that the time taken to produce a target molecule can be reduced by up to eighty percent, allowing project delivery timescales to be reduced accordingly. In a normal chemical laboratory the chemists multitask to make the most efficient use of their time, but this does not reduce the actual time taken to move from molecule concept to a synthesized, purified target molecule. The Biotage automated workflow addresses this directly with methodologies expressly designed to reduce the time taken from target identification to delivery of purified product.

Introduction

The spiraling cost of pharmaceutical R&D means that pressure to work smarter in drug discovery has never been greater. The cost of launching a drug on to the market has recently been estimated at $2.7 billion[1], over fifty percent of which is accounted for in investor losses while research into the new drug is on-going. Identifying and completing research into that new drug is principally an act of finding and dismissing unsuitable molecules from the billions available in the chemical research space until the right molecule is found—on average it is estimated that between 5,000 and 10,000 potential drug molecules are investigated to produce one approved therapy[2]. The huge cost of drug discovery has forced new paths for R&D, with CROs and outsourcing becoming increasingly competitive alternative models to traditional pharmaceutical R&D.

The potential profitability of drugs is also influenced by their development time, as it takes on average 10 years to move a molecule from the discovery stage to an approved drug on the shelves, and the twenty year patent exclusivity period begins when human trials are underway.

In the laboratory, the integrated approach to drug discovery presents the laboratory head with a target list of potential molecules with required physio-chemical parameters that must be made to the necessary purity within as short timeframe as possible. Chemists multitask to ensure their time is used most efficiently, but the process of delivering the appropriate library by creating molecules on the target list is still time consuming and a potential bottleneck to project progression. Longer timescales in synthesis also restrict chemists from a more agile approach to projects, reducing flexibility of the laboratory to take on new projects.

The Biotage Automated Workflow Solution

Reducing the bottleneck of target list synthesis is possible through the adoption of the Biotage automated workflow solution into your laboratory. Utilizing a range of interconnected automated platforms, the workflow is designed to significantly cut the time taken to produce a target molecule of sufficient purity. The Biotage automated workflow solution is based around three technologies that interconnect to reduce the overall time to target molecule:

1. Synthesis using microwaves to drastically reduce overall reaction time.
2. Purification of reaction mixtures using an automated flash system with high-capacity columns and reduced runtime methodologies.
3. Evaporation of solvents from fractions using a rapid, automated evaporation system.

In combination in an automated approach, these technologies vastly speed the time taken to go from the concept of a target molecule to the final product. Each of these technologies shall be discussed in detail below.

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Microwave Synthesis

The Arrhenius equation determines that for a chemical reaction a ten degree rise in temperature doubles the reaction rate, and so traditional chemical synthesis often involves heating samples to high temperatures. Unfortunately, even with conventional heating, reactions can be very long with reflux times of many hours. Obviously this is a barrier to the rapid synthesis of target molecules for further investigation. In contrast to conventional heating, microwave irradiation is becoming a well-established method of increasing the reaction rate of chemical processes as elevated temperatures can be achieved quickly and under a high level of control under pressure, allowing temperatures in excess of the boiling point to be safely achieved. Using microwave assisted organic synthesis (MAOS), a reaction that might originally have taken hours can be achieved in a few minutes, with increased reaction efficiency and therefore compound purity at the same time. Two examples are shown in figure 1 with comparison to conventional synthetic routes—hydantoin synthesis and an epoxide ring opening reaction. In both cases, the MAOS route is significantly faster than the traditional approach.

Most microwave systems are batch designs, allowing sequential reactions to be performed under identical, carefully controlled conditions, ideal for combinatorial chemistry approaches. However, these systems are also ideal for the rapid synthesis of a target molecule, as complex multi-stage synthetic procedures can be performed rapidly. An example is the synthesis of Fluoxetine Prozac, which can by synthesized in 3-steps starting from 2-chloroacetophenone as shown in Figure 2 and Tables 1 and 2. The total time taken to synthesize fluoxetine using microwave heating is about 12 minutes vs. the 1 day of conventional synthesis with comparable overall yields.

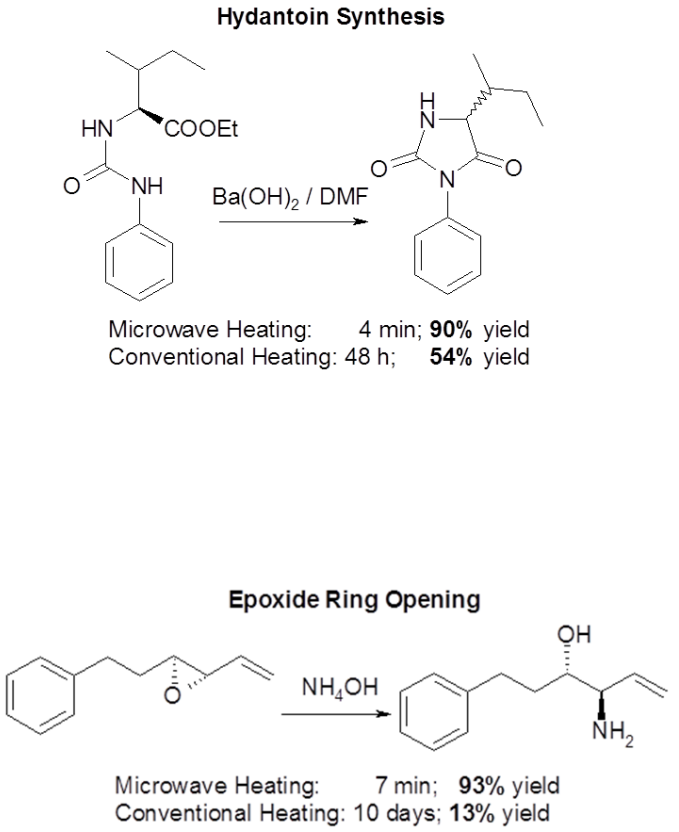

Figure 1. Hydantoin synthesis and epoxide ring opening with conventional and microwave heating.[3, 4]

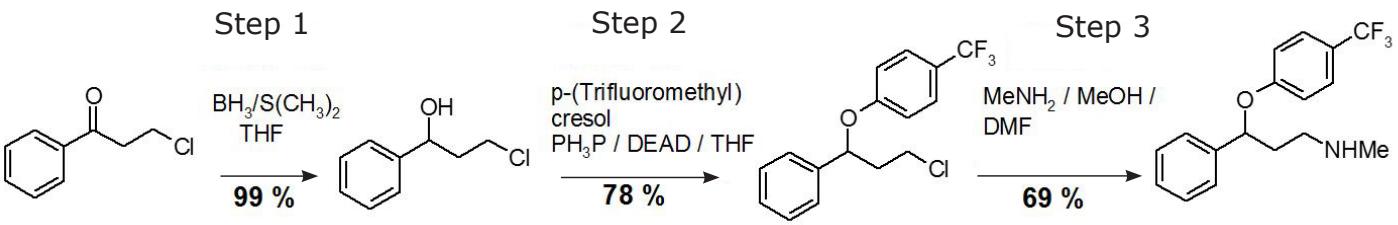

Figure 2. Synthetic route to Fluoxetine Prozac.

Conventional Heating

Step	Temperature (°C)	Time	
1	-25	7 hours	
2	23	18 hours	
3	130	3 hours	
	Totals	28 hours	51% Yield

Table 1. Reaction times with conventional heating.

Microwave Heating

Step	Temperature (°C)	Time	
1	100	2:20 min	
2	120	5 min	
3	150	5 min	
	Totals	12:20 min	53% Yield

Table 2. Reaction times with microwave heating.

Automated systems allow the manipulation of reactions, which can be queued to run sequentially without interaction from the user. As a result microwave assisted organic synthesis (MAOS) may be employed to trial new reactions and rapidly develop new molecules on the small scales required for preliminary screening, and microwave heating has become an important strategy in increasing the speed to success of a synthetic process.

Figure 3. Biotage® Initiator+ Microwave Synthesizer with Robot 60.

Automated Rapid Flash Purification

Traditional purification of molecules post-synthesis involves self-packed glass columns containing silica, typically normal phase. Elution is performed by gravity or with the assistance of compressed air after the sample is introduced to the column via a syringe. This is known as flash chromatography. Automated flash chromatography systems make use of pre-packed flash columns and solvent delivery pumps to greatly speed the process, and detectors coupled to fraction collectors to automated recovery of sample at the end of the process. Automated flash systems give orders of magnitude time savings over glass columns, which can take many hours to complete as is illustrated in table 3.

	Glass Column	Automated Flash System
Typical Flow Rates	1–10 mL/min	25–100 mL/min
Average Preparation Time	30 minutes	5 minutes
Typical Run Time	0.5–2 hours	5–15 minutes

Table 3. Comparison of typical purification methods on a glass columns and an automated flash system.

The Biotage automated workflow makes use of the Biotage® Selekt Flash purification system. This instrument has been designed with speed and efficiency in mind, with the smallest footprint of any automated flash system and two built-in column channels to run two separations on the same instrument. The Biotage® Sfär columns have the highest loading and the lowest solvent consumption. Figure 4 shows typical flash runs for a four component mix on Sfär and Sfär HC columns.

Sfär HC columns, where HC stands for High Capacity, have twice the loading capacity of standard Sfär and other commercially available columns. All Sfär columns can be operated at high pressure ranging between 12 bar and 20 bar, allowing the Selekt system to perform rapid pressure-limited column equilibrations. Coupled with the high separation flow rates possible on Sfär and the exceptional loading capacity of both Sfär and especially Sfär HC, the Selekt and Sfär combination is the fastest and most efficient method of flash purification.

Biotage® Selekt four component mix

- **Sample** methyl paraben, propyl paraben, quinoxaline, and 4'-methoxy acetanilide in acetone
- **Solvent A** hexanes
- **Solvent B** ethyl acetate
- **Gradient** 7% B for 1 CV
7–50% B in 8 CV
100% B for 3 CV
- **Detection** λ-all 200–400 nm, 254 nm, 280 nm
- **Load** 0.1 mL (30 mg)

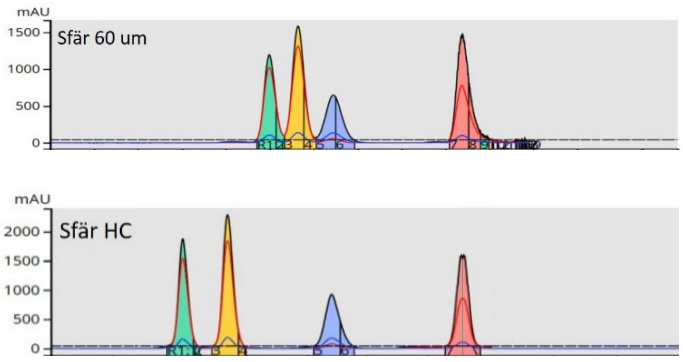

Figure 4. Column performance with a 4-component mix.

A fully automated flash purification system is able to automatically fractionate a complex reaction mixture into individual components in a very short timeframe. Detection of molecules eluting from the flash column can take on many forms from a simple UV to a evaporative light scattering detector (ELSD) depending on the level of information required from the sample. This allows the flash system to replace more time consuming but high resolution or information rich approaches in some cases, for example preparative HPLC.

Sample Evaporation

Recovery of the final sample after purification is one of the most time consuming parts of small-molecule synthesis, especially if the sample is contained in a high boiling solvent which is difficult to remove, such as water. The traditional approach would be to use a rotary or centrifugal evaporator similar technology, which takes several hours to remove difficult samples and requires constant attention from the user. Alternatives such as freeze drying are also very time consuming and often require a critical number of samples before they can be employed.

Within the Biotage automated workflow, evaporation is achieved using technologies specifically designed to allow the easy removal of high boiling solvents in very short timeframes, the Biotage® V-10 Touch evaporator. This approach makes use of three factors to drastically reduce the time taken to remove solvents:

1. Sample is spun in a vial to form a thin film of maximum surface area to aid evaporation and remove the possibility of sample 'bumping'
2. Heat is applied uniformly to the spinning sample
3. Vacuum is applied

All of these factors can be controlled independently to ensure the best evaporation conditions, resulting in extremely short evaporation times as shown in table 4, especially in comparison to alternative techniques.

Automation is achieved through the use of a carousel to automatically load and unload samples, and a liquid handler to dispense solutions from one container to another, such as when concentrating down a large volume of sample into a small vial.

Result of Adopting the Biotage Workflow Solution

Using the Biotage workflow solution as opposed to traditional methods, it is possible to achieve significant time savings on route from molecular target to final delivered product. This is summarized for an average reaction in table 5, assuming that the route has five synthetic steps.

Figure 5. Biotage® Selekt with UV-VIS detector

Solvent	BP (°C)	Biotage® V-10 Touch Evaporation	Centrifugal Evaporation	Blow-down Evaporation
NMP	202	18 minutes	N/A	12 hours
DMSO	180	15 minutes	180 minutes	N/A
DMF	150	4 minutes	90 minutes	N/A
Pyridine	115	5.5 minutes	70 minutes	N/A
Water	100	9 minutes	140 minutes	240 minutes
Methanol	65	3 minutes	70 minutes	40 minutes
Cyclohexane	81	2.5 minutes	40 minutes	20 minutes

Table 4. Time to evaporate 8 mL of solvent at the appropriate pre-set method.

	Synthesis Methodology			
	Traditional		Microwave	
Setting Up Reaction	60	minutes	60	minutes
Perform Reaction	480	minutes	5	minutes
Workup of Reaction	60	minutes	60	minutes
	Purification Methodology			
	Glass Column		**Automated ACI™**	
Prepare Column	60	minutes	15	minutes
Run Column	60	minutes	15	minutes
Identify Fractions	60	minutes	0	minutes
	Isolation Methodology			
	Rotary Evaporator		**Biotage® V-10 Touch**	
Evaporate Sample	15	minutes	5	minutes
Summary				
Total for one Step	795	minutes	160	minutes
3 Step Synthesis	2385	minutes	480	minutes
In Hours	39.75	hours	8	hours
Relative Time	100%		20%	

Table 5. Comparison of the time taken to prepare a target molecule in five steps using a traditional approach and the Biotage Automated Workflow.

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Summary

The Biotage automated workflow solution delivers target molecules in on average a fifth of the time of traditional chemistry. The Biotage automated workflow is also built around greener chemistry, significantly reducing the consumption of chemicals. As a result, adoption of the workflow produces a laboratory with a significantly improved environmental profile in terms of carbon footprint, with accompanying reduction in purchasing, storage and disposal costs. A single automated workflow with one provider means that downtime is reduced, as service and preventative maintenance can be scheduled across the entire workflow. Utilizing the Biotage automated workflow allows the laboratory to become more flexible to incoming projects and changes in demands on resource. This ensures that the laboratory remains competitive with other recipients of R&D funding.

References

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Why Should Your Laboratory Adopt the Biotage Automated Workflow?

There are many reason why adopting the Biotage automated workflow might work for your organization. Despite how much time is spent studying the literature for new ways to make interesting molecules, many chemists, even in a time-critical results-driven environment, are still relying on chemical techniques from the previous century. The Biotage automated workflow uses state-of-the-art technologies to ensure that your processes are as up-to-date as your thinking, putting you in the top tier of medicinal chemistry companies in target delivery. Many companies are adopting environmental commitments for greener chemistry that have a large impact on the synthesis laboratory. These regulatory-driven changes mean that the traditional ways of producing target molecules are no longer acceptable, and new methodologies must be established for the future. The Biotage automated workflow has greener chemistry built-in, meaning that adoption of the workflow is not only improving your efficiency but also future-proofing your laboratory against increasing environmental demands. Your company has many ways to approach R&D, including outsourcing to external partners such as CROs. Internal medicinal chemistry laboratories need to ensure that they represent the best value for money for the R&D spend allocated to them. The Biotage automated workflow solution gives your laboratory the fastest response time to target list requests and places you in a position to deliver in the most time efficient manner.

Bring the Biotage Approach to Your Laboratory

To learn more about how the Biotage Automated Workflow approach, visit www.biotage.com.

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