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Preparative LC/MS Technology: A Key Component of the Existing High Speed Synthesis Platform at Syngenta

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Abstract: Sizable investments in laboratory automation and state-of-the-art purification equipment were made at Syngenta Crop Protection with the objective to increase synthesis capacity and effectively support typical lead optimization activities. The successful implementation of a *high-speed-synthesis* platform consisted of the modular assembly of various commercially available liquid-handling workstations in combination with in-house developed parallel synthesis reaction manifolds. Preparative LC/MS technology was identified as a key success factor for optimizing overall throughput by addressing existing bottlenecks in parallel synthesis such as the fast and unattended isolation of compounds. Automated data analysis is crucial for high-throughput quality control and to allow all compounds of interprets to be sorted rapidly, *i.e.* those having defined structures and passing predefined purity criteria. The integrated application of the tools as described is intended to support chemists in their task to derive reliable structure–activity relationships more quickly and hopefully to shorten the time needed to identify innovative drug candidates.

Keywords: Automated synthesis · Combinatorial chemistry · laboratory robotics · LC/MS · Preparative HPLC

Combinatorial chemistry was invented in the late 80's as a powerful new method for probing structure–activity relationships [1]. Its importance today can be demonstrated by the size of the investments most major pharmaceutical and agrochemical companies have made in order to implement combinatorial technologies into their own research strategy. Major driving forces were, and still are, the desire to find structurally novel drugs and to shorten the drug discovery cycle in general [2].

Over the years a number of ingenious concepts and different techniques were described in the literature, ranging from manual and truly combinatorial synthesis on solid support [3], towards parallel and more automated approaches, favoring the liquid phase [4]. As a result of these developments today's researcher can make use of a new set of tools, the selection of which largely depends on the characteristics of the problem to be solved. At least two major fields of application can and should be distinguished in drug discovery: lead finding and lead optimization. While the former is engaged in the art of selecting, designing and preparing novel compounds with highly diverse properties, lead optimization is primarily interested in the fast exploration of structure-activity relationship around a novel hit (Fig. 1). It is probably fair to say that the majority of solid-phase applications today are used in connection with (*de novo*) lead finding activities, while solution-phase based approaches, supported by varying degrees of laboratory robotics, seem more geared for lead optimization activities.

The clear strategic focus at Syngenta Crop Protection on 'lead optimization' has led to the construction of a highly effective *high speed synthesis* platform based on solution-phase chemistry and characterized by the extensive use of laboratory robotics. The implementation process started a couple years ago with a

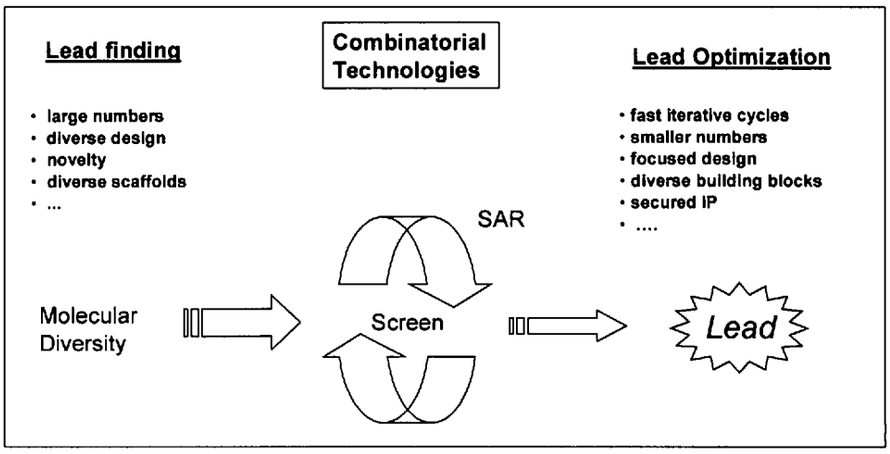

Fig. 1. Impact on drug discovery by combinatorial technologies.

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workflow analysis in parallel synthesis and led to the conclusion that not the synthesis process itself was considered the potential bottleneck, but rather typical post-synthesis activities such as analytics, purification and data handling in general (Fig. 2). The result of this analysis made the optimal allocation of sizable investments clearer and had direct implications on the guiding principle in selecting equipment as well, *i.e.* the automation of all the typical synthesis activities was addressed with cheap and rather low-tech devices, while the automation of the downstream activities involved considerably more expensive and state-of-the-art methodology from the very beginning.

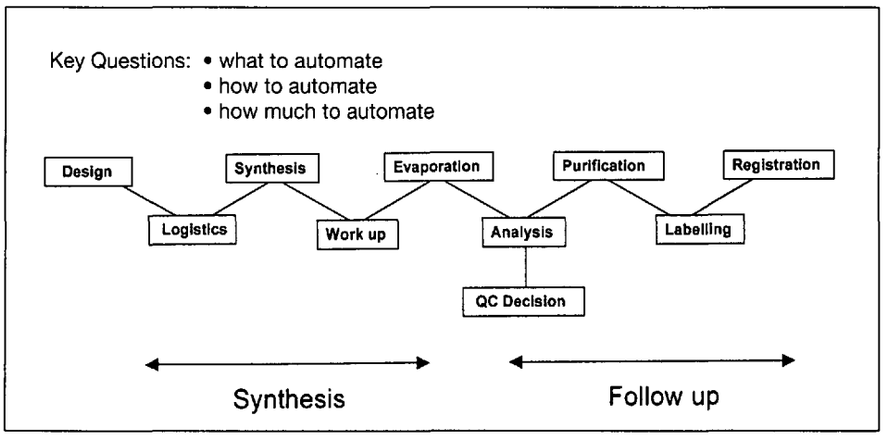
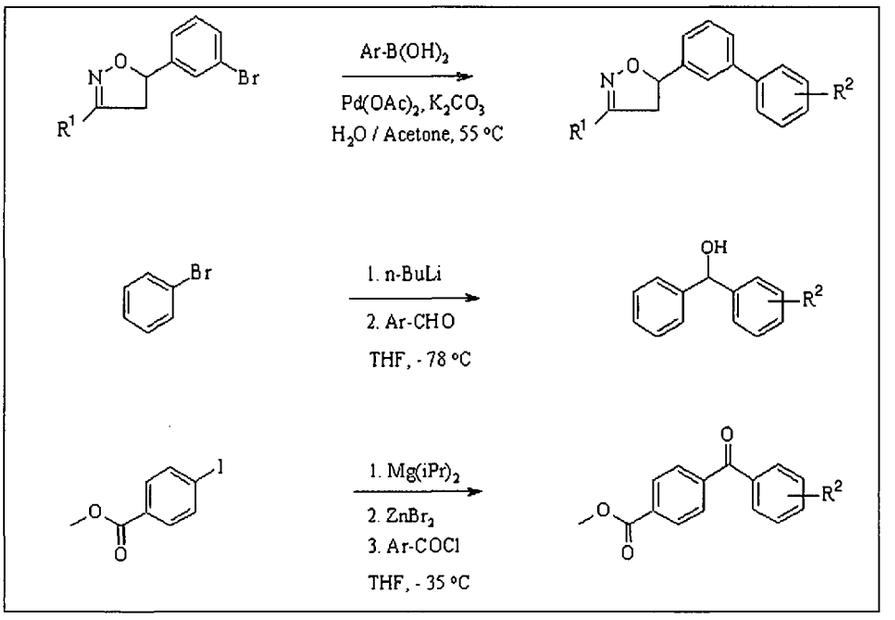


Fig. 2. Workflow in parallel synthesis.

The combination of custom-designed reaction manifolds, manufactured from aluminum and based on a microtiter footprint, with commercially available liquid-handling robots in a modular automation concept as described previously [5], allows the operation of several *high-throughput synthesis* laboratories with rather small investments. This rigorously applied workstation approach involves by definition some manual intervention steps, *i.e.* it is not fully automated, but offers the advantage that identified bottlenecks or future technical developments are easily addressable without changing the whole infrastructure of the production lines. What might be surprising at first glance is the wide range of chemistry that is applicable to automated parallel liquid-phase synthesis in these rather primitive reactor blocks. Even the processing of quite delicate metal-organic reactions is feasible without much sacrifice on synthesis fidelity, as is depicted in the Scheme.



Scheme. Chemistry examples (performed).

Remaining problems in parallel synthesis clearly have their roots not in poor reactor design but are rather an intrinsic characteristic of the combinatorial approach itself, in other words, they are the result of the quite different physical and/or chemical properties of sets of maximally diverse reagents forced to react under only one given reaction condition. Unless a significant amount of time is spent by a skilled chemist searching for the optimum reaction conditions, the result of parallel synthesis experiments are often the transformation of reagents into reaction mixtures containing quite variable amounts of the desired products, *i.e.* a decision has to be made between targeting maximum diversity and increased speed resulting from parallel handling. We think that a partial way out of this dilemma exists in the form of extensive application and automation of chromatographic methods for reaction clean up and product isolation. And we believe that it is in this area that a robust hyphenated technique like LC/MS, which enables the simultaneous separation and identification of compounds, has a unique strength and can make crucial contributions to the overall efficiency of the parallel synthesis concept.

What follows is a short description of the principle of preparative LC/MS technology, an invention made a couple years ago by Weller *et al.* [6] in response to what was back then the 'purification bottleneck' in combinatorial chemistry. It is a beautiful example of how technological progress can significantly speed up tasks performed by chemists in the drug discovery industry for decades, namely the isolation of compounds whose molecular weight is known upfront. Preparative LC/MS technology became possible with the idea of using a post column flow splitter and (destructively) analyzing in real-time the minor-flow stream by a modern mass spectrometer (ES-MS). This on-line monitoring enables a fraction collector to be triggered once the mass of interest is detected above a certain threshold and therefore allows compounds to be isolated selectively out of the remaining (and delayed!) major fluid stream. Fig. 3 shows in detail the wiring diagram and the actual hardware/software configuration of systems currently in use at Syngenta and serves to better illustrate this intelligent fraction collection process.

Because an on-line decision-making process is involved in compound isolation, it is self-evident that the reliability of the splitting process and the timing of the trigger signal are absolutely crucial in order to not lose samples of interest. The benefit of the set-up just described lies in

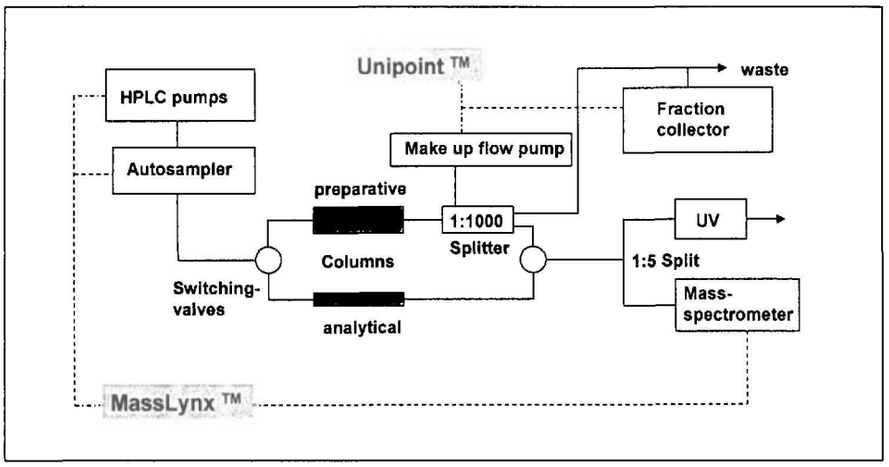


Fig. 3. Wiring diagram LC/MS.

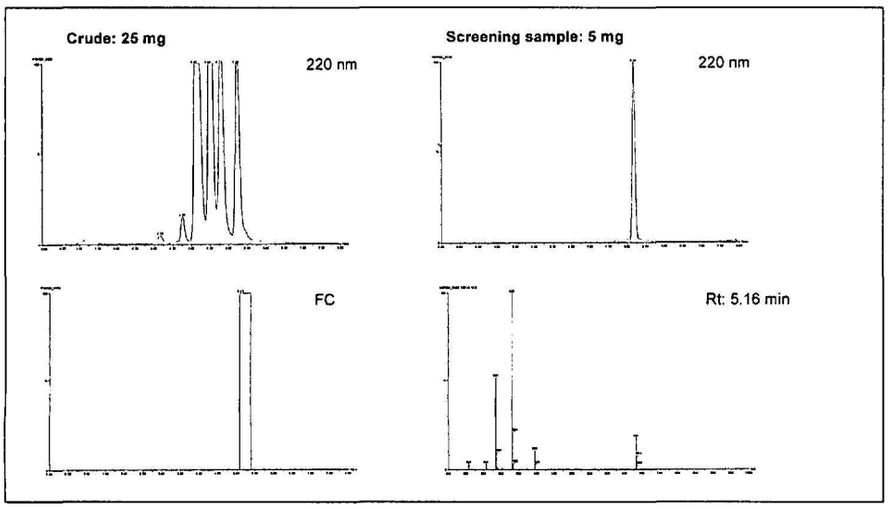


Fig. 4. Purification example.

the capability to isolate on a single instrument up to 200 pure compounds/day in multimilligram quantities without losing track of the fraction locations or being overwhelmed with structure assignment. And batch processing of hundreds of compounds, not an untypical task for combinatorial chemists, is easily possible due to the ease of electronic data transfers and automatic data assessment by an in-house developed VB macro as described elsewhere [7]. The kind of purity enrichment and information content one can expect from such systems operating in *high-throughput* mode under RP conditions, using a 6 min gradient, is shown in Fig. 4.

It is true that preparative LC/MS systems are not cheap by traditional chemists standards, but a sober analysis about how much time is traditionally spent in most organic laboratories in purification efforts reveals immediately the huge potential for a fast return on the capital investment. And because pure compounds with assigned structure are a prerequisite for establishing reliable structure–activity relationships, such purification platforms have become a key success factor in making the drug discovery process more effective.

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