Right by design

Design of Experiments has far wider use than simply optimising yields, says **Dr Jacobo Cruces** of **Galchimia**

Design of Experiments (DOE) is probably one of the most powerful tools available to organic chemists.[1] Basically, it can be applied to three different problems: screening, where the aim is to find and identify influential factors; optimisation, looking for the best combination of previous factors; and, robustness testing, where the aim is to determine how sensitive a product or process is to small changes in the factors.

However, although DOE has made its way into the development part of the pharmaceuticals and chemicals industry when applied to optimisation and robustness testing, it is still underused by researchers to tackle screening problems, especially in the drug discovery field.[2]

Let us remember that drug discovery is about the number of products, while process development is about the process, since the product is fixed. Most discovery chemists perceive that, since DOE is mainly used to improve manufacturing and development aspects, which in chemistry means yields in 99% of cases. Therefore, they tend to see it exclusively as a tool to optimise the yield of a reaction.

In the discovery phases of a project, the initial requirements of volume of product, purity and others are fixed from the outset, but if you have a new product in sufficient quantity to perform the first activity assay, the rest can be negotiated. Therefore, any effort during the drug discovery phase to optimise or improve the chemistry is usually perceived as a waste of time, rather than an investment which can pay benefits in the short term.

This vision of the possible benefits of DOE is often based on ignorance about its principles. DOE provides an organised approach to experimental work, which is a strong point when tackling complex research projects. The chemist should select the experimental objective, then the methodology guides the design of a proper set of experiments to obtain the required information.

Moreover, the experimental objective for a discovery chemist is not necessarily the best yield of a given product. The yield can be the objective or just an adequate response factor to evaluate how some variables influence the outcome of a reaction.

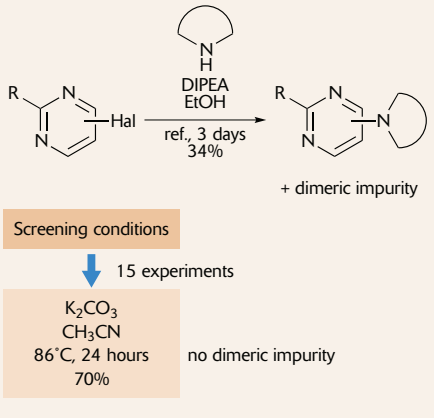

Figure 2 - DOE for the regioselective palladium-catalysed arylation of 4-chloropyrazole

A typical example is a reaction where two isomers, A and B, are obtained. The ratio between them can be used as a measurement but the yield of A can also be used. In such a case, a better idea is to gain some understanding of the factors influencing the system under study, which will surely lead to an optimised yield in the end.

Many articles are published every year related to the development of new reaction conditions, most of them following the intuitive approach of changing one factor at a time (OFAT). However, the DOE approach handles three critical issues more efficiently than OFAT.

The first problem is precisely the understanding of systems influenced by several factors. OFAT fails because changing only factor at a time does not allow one to estimate the interaction between factors.

Secondly, when using OFAT the systematic and unsystematic variability - that is, effects and noise - are very difficult to estimate. Thirdly, the response contour plots of systems are hard to produce if a DOE approach is not used. These allow for the prediction of reaction conditions, which is, of course, a very interesting tool for any chemist.

A very recent example in the literature published by Lilly chemists illustrates the concepts given above.[3] The authors state that, in connection with a drug discovery programme, they required a methodology to access 5-aryl-1-methyl-pyrazoles quickly. That is, they needed not the best yield, but a method delivering the desired isomer. The reaction under study was a metal-catalysed arylation of 4-chloropyrazole (Figure 1).

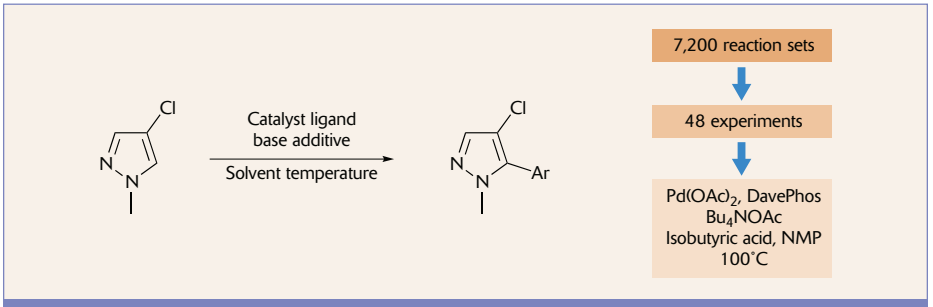

Figure 1 - DOE for the regioselective palladium-catalysed arylation of 4-chloropyrazole

Five factors were to be studied: solvent, base, catalyst, ligand and additive. For these factors the authors, based on their previous expertise and the information available in the bibliography, contemplated six, ten, four, six and five possibilities respectively. To explore each set of conditions a total of 7,200 experiments would have to be done.

With DOE and statistical software, the number of experiments was reduced to 48 and, even with so few experiments, statistical analysis yielded useful information. JMP by SAS was used in this instance but many other valid statistical packages are available, such as MODDE by Umetrics and DesignExpert by StatEase.

This example highlights the benefits of a DOE approach in comparison with the traditional approach. Although some chemists might argue that the final conditions are not so different from the solution they would have provided under the current knowledge for these C-H activations, there are several points that must be emphasised.

Firstly, DOE offered a rational, organised approach to the problem. In addition, the experiments done and the subsequent analysis show not only that Bu_4NOAc is the best base but also that the other four factors are of low relevance to the outcome of the reaction. Finally, the analysis predicted two different sets of conditions that were not previously performed in the 48 reaction set.

It should be noted that the OFAT approach is usually found in academic papers, while the DOE approach often seems to be found in industry papers, especially those related to scale-up and process development.[4]

As noted before, since a drug discovery project is driven by the need to find the best product and the best way is to prepare many different compounds, any DOE effort appears useless, unless you are faced with a problem like the one described above. However, the information and understanding gathered through a DOE study might be of value, not only for that specific project but maybe for other similar projects and even in the later phases of the discovery process if a lead compound with such a structure is chosen.

There are additional arguments to support favouring the DOE approach in early drug discovery phases. If the key to drug discovery means investing time to prepare compounds, any inefficiency that leads to time being wasted should be corrected.

A typical case is critical reactions where the outcome is a mixture of isomers and starting materials. Medicinal chemists complain, sometimes bitterly, about the amount of time wasted performing tedious purifications, with enormous quantities of starting materials being used to obtain only a few grams of the required scaffold. Even a partial DOE effort can improve the reaction conditions and increase productivity.

An example of such improvement carried out by Galchimica illustrates this concept.[5] In the framework of a drug discovery project, we were preparing a heterocyclic scaffold derived from a chloroheterocycle by reaction with a high value amine (Figure 2).

Although the reaction worked, it was slow, conversion was low and a dimeric impurity was present in significative quantities. These problems translated into low yields (34% after three days) and difficult purifications. We encouraged our client to allow us to improve the reaction, which in turn would be returned to them so that the benefits could be applied to the synthesis in progress.

Time was of paramount importance, so a complete DOE optimisation was ruled out. A quick bibliographic search showed that similar reactions had been described using metal couplings, but not over our scaffold. In order to avoid longer development times, metals were discarded. Using microwave conditions was an obvious improvement but this was also discarded to avoid scale-up issues.

We therefore concentrated our efforts on finding a new combination of solvent and base, so we designed a fast screening evaluating nine different solvents and two bases, using as response factors the percentage of starting material and product obtained by LC-MS. Not all of the 18 possible combinations were considered, since some hints available in the literature led us to expect bad results from some combinations. From the first results obtained an additional set of improved experiments was carried out.

The second batch of experiments allowed us to develop a set of improved conditions. The reaction time was shortened from 72 hours to 26, while yield improved from 34% to 70%. Even more importantly, although conversion was only 87%, with 12% recovery of the starting material, no dimeric compound was obtained, which made the purification easier and faster.

This demonstrates that even a handful of experiments (a total of 15 reactions were run, including a reproducibility check) under time and technology restrictions (no full optimisation, no metal-mediated couplings, no microwave heating) following the DOE guidelines make a difference. The time saved could be invested in more productive tasks.

Summary

The implementation of DOE in the early stages of the drug discovery process leads to enhancements in productivity by reducing time and costs wasted in low value tasks. As an added benefit, the true understanding of factors influencing a given system can be used during the scale up and process development stages.

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