focus on
Chromatography

From Crude Mixture to Pure Compound - Automatic Purification Made Easy

Dr Sascha Giegold, HPLC Product Specialist, Shimadzu Deutschland GmbH

The development of active substances is driven by high-level throughput, effective-ness and efficiency, but in equal measure product quality, reliability and ability for reproduction is required. Therefore, mostly automated technologies support these demands. The new Crude2Pure system (C2P) simplifies the purification process after preparative LCMS and offers an easy method to generate for example lead structure candidates by defined salt form and high purity already in the early development. It utilises a 'Trapping Module' and a new recovery technology to receive the active sub-stance possibly as a solid. The 'Smart Cap' of the test vessel supports reproducible results, while the Open Access structure enables to control the C2P as a whole from several labs.

The production of active substance candidates in the early development stage is carried out in several steps. Particularly, the purification is often the most time-consuming and sophisticated step to receive the desired salt form in high purity. To simplify and automate these processes, the C2P system was developed. On basis of a liquid chromatography system (HPLC), the substance coming from the preparative LCMS is loaded on a cartridge, where all HPLC background is removed, and the target molecule is transferred into the desired salt form. Afterwards, the cleaned substance is sprayed in a vessel to receive a highly purified compound.

Automation in API Research

The aim of automating the drug development process is to increase the efficiency which means, on this occasion, not only throughput per employee, but also an increase in the quality of the product and the reliability and ability for reproducibility of the processes. However, most substances that come from lead structure discovery have been isolated by means of HPLC and investigated as amorphous solids. Nevertheless, for pharma kinetic (PK) studies it is indispensable to use a defined salt form, so that a suitable formulation can be found for the administration [1]. Therefore, the need insists in an automated method on generating a leading structure candidate by defined salt form and high purity already in the early drug development, and the method should be highly reproducible to always produce the same product quality.

The Crude2Pure System

Exactly at that point, the C2P system begins. The C2P system consists of a trapping system equipped with a dedicated Shim-pack C2P-H column for trapping and concentrating the target compound, and a recovery system that automatically powders and recovers the trapped target compound. It is suitable for a wide range of molecules as depicted in *Figure 1*.

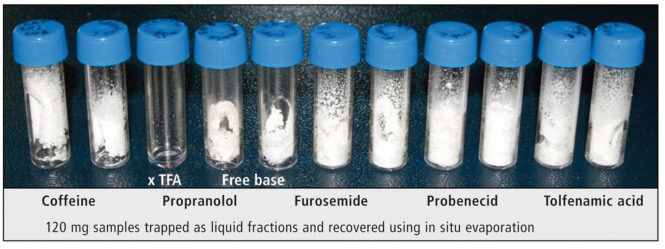

Figure 1. Example of different molecules trapped and recovered by Crude2Pure

The Trapping Module

Ordinarily, when removing solvents, it is necessary to evaluate detailed conditions for each compound. Applying the C2P, however, the users simply place the preparative LC fraction in the autosampler, and enter the compound information into the interactive software to automatically trap the compound. In addition, the newly developed Shim-pack C2P-H trapping column can be used with a wide range of organic solvents and pH conditions, and provides high loading and high recovery rates. Furthermore, trapping conditions are determined from the information on retention times obtained during fractionation, so there is no need to evaluate conditions individually. This eliminates the need to perform difficult preliminary evaluations to determine trapping conditions.

The Recovery Module

This stage recovers the target compound trapped on the trapping column and then powders it simultaneously. In addition, it can perform desalination and salt substitution as is, prior to recovery.

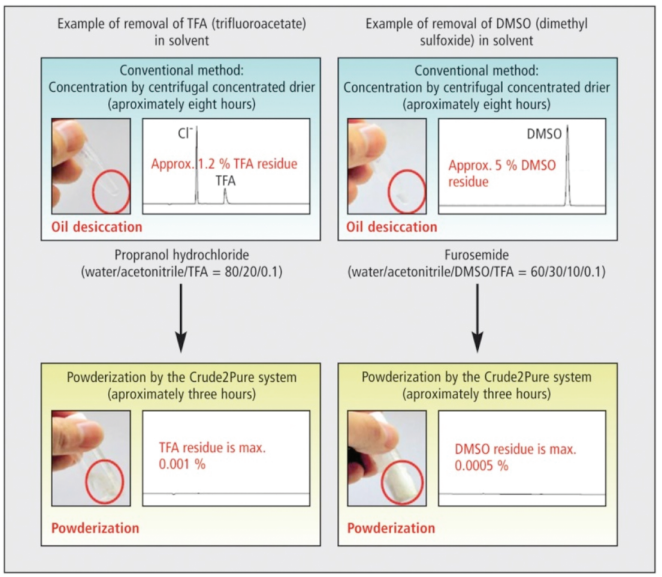

Figure 2. Example of removal of TFA (trifluoroacetate) and DMSO (dimethyl sulphox-ide) in solvent

In solvent removal with a centrifugal concentrator, which is a conventional method, it is difficult to remove solvent if the mobile phase includes components which generate counter ions, such as trifluoroacetate or difficult volatile components, for example dimethyl sulphoxide. The C2P system easily removes components included in fractionated liquid from the preparative LC system and recovers the sample as a powder *(Figure 2)*. By method selection at the time of recovery, it is possible to remove mobile phase components and make powder as free-base.

The sequence from pretreatment to powder takes approximately three to four hours, which saves a great deal of time in comparison to centrifugal concentration or freeze drying *(Figure 3)*.

The vial in which the powder target compound is recovered can be used as a storage container as is, eliminating the need to transfer the samples. Furthermore, adding the sample rack changer option increases the throughput of drying and powder via six-line parallel processing.

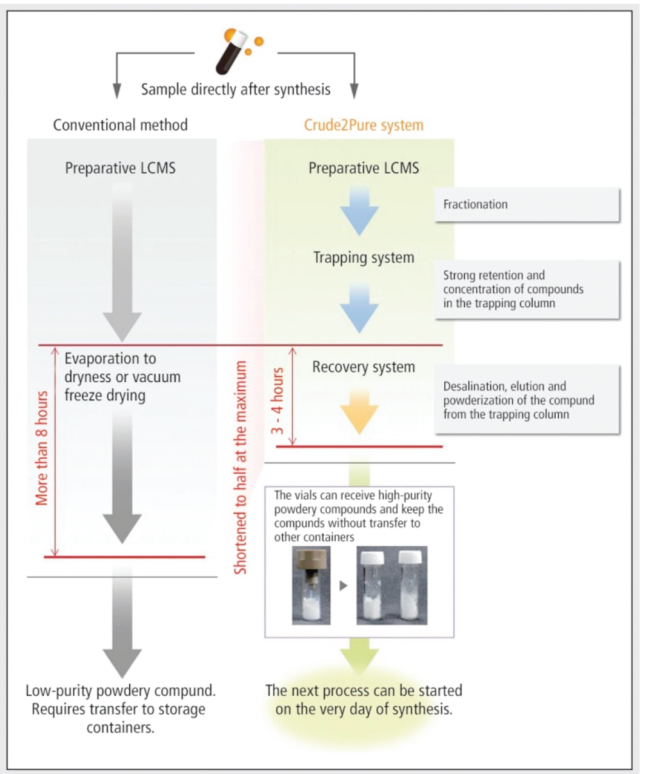

Figure 3. Comparison of conventional purification process with Crude2Pure System

Conclusion

With all its advantages – including high levels of reproducibility created by the automation of all necessary steps in the purification of active substances, the high purity of the products yielded through this process and not least the simple operation and acceleration of the work routine - the C2P platform delivers an important contribution to the development of new active substances. Thus, the first drug discovery studies become more expressive, because highly pure substances can be used and therefore the influence by impurities can be nearly excluded from the efficacy of a molecule.