

CASE STUDY

Combined LC-MS / LC-SPE-cryoNMR method applied to drug impurity identification

Drug impurity issue

The identification and quantification of impurities in Active Pharmaceutical Ingredients (API) and Pharmaceutical Products is a very intensive activity performed at many levels of the drug discovery pipeline and beyond (see **Figure 1**). Impurities relate to starting materials, by-products, breakdown products or polymorphs. They can appear at the API production level as well as during or after the formulation process. Impurity concentrations may change upon storage of the product. Impurities in APIs are of significant concern as they may carry activity responsible for eventual undesirable side effects or toxicity and/or may interfere with the drug's activity. Thus monitoring impurities in API and Drug Products is a prerequisite to insure drug safety and quality. Therefore specific requirements for impurities are set by the regulatory authorities when submitting a new drug application (see e.g. [FDA ANDA guidelines 2009](#)).

The monitoring and understanding of occurrence of impurities need to be addressed at a very early stage of development of a small molecule. It will indeed have a significant impact on the researched activity, production process, formulation, or even on the drug delivery. It is sometimes not economic or technically feasible to remove all impurities during the production process, so they need to be monitored. Introducing a compound to the market will always be accompanied with an *impurity profile* to guarantee that the

product's quality matches with the specification filed at the regulatory authorities (e.g. FDA, EMEA).

Regulatory organizations (ICH, FDA, EMEA) have defined a standard threshold above which impurities have to be properly identified (>0.1% of the API). Identification of these impurities are classically done by LC-MS(/MS) and sometimes supported by NMR analysis, when their concentrations level is accessible. However, despite the diligence and experience of (analytical) chemists, in a significant number of cases, it remains very difficult or impossible to establish the identity of these impurities. For instance, MS conditions could be incompatible with the stability of the molecule, the isolation of the impurity in pure form may happen to be very difficult, or the structural data collected could just be insufficient to generate an hypothesis regarding the impurity's identity.

Spinovation solution to drug impurity identification

Spinovation Analytical has available the most powerful **integrated analytical solution to impurity identification**, able to tackle virtually any impurity issue. Applying LC-SPE-NMR methods, we ensure fast and efficient isolation of the impurities of interest and get access to high content structural data (NMR) on mass limited compounds. Using an integrated NMR MS approach we derive the data to determine the structure(s) of the impurities with the highest level of confidence.

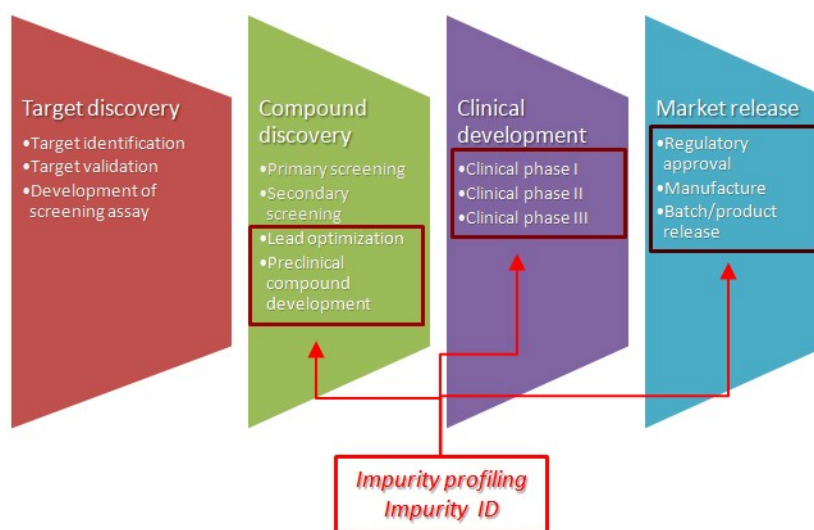


Figure 1. Drug impurity profiling / identification activities are carried out all along the drug discovery pipeline and beyond.

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Case study: identification of 2 API-related impurities unstable under LC-MS conditions.

In a tablet formulation of an API submitted through forced UV exposure, 2 unknown impurities were detected at a level > 0.1% (as compared to the main component). LC-MS analyses revealed that these 2 impurities were related to the API and that they were characterized by the same mass and fragmentation pattern. However, despite the effort of the client's chemists, the identity of these 2 impurities could not be established.

At Spinovation, we applied a LC-SPE method to isolate and pre-concentrate the individual impurities. Upon control of the LC-purity of the 2 isolated compounds, we established that they were inter-converting isomers, within a ratio of about 30:70 when equilibrium was reached (see **Figure 2**).

Applying LC-SPE-cryoNMR method, we identified ^1H -NMR resonances characteristic of anomeric protons (see **Figure 3**). The use of SPE also allowed us to get a better insight into the chemical exchange process that was taking place, as the SPE step helped to slow down the exchange [impurity 1 \leftrightarrow impurity 2]. Indeed, using direct transfer from SPE to NMR, we were able to observe the conversion before the final equilibrium could be reached.

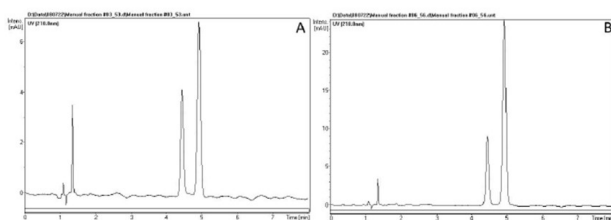


Figure 2. Chromatograms of the 2 impurities analysed by LC-UV after being isolated in pure form. In case of impurity 1 and 2 (chromatogram A and B, respectively), both impurities are detected supporting inter-converting events and that they are likely to be isomers.

Integrating the LC-UV, MS and NMR data we derived the structure of the 2 impurities being respectively the α and β forms of hemi-acetal isomers. These hemi-acetals were very unstable in MS conditions leading to dehydration and rearrangement (100% conversion), so

that a structure only based on MS data could not be derived.

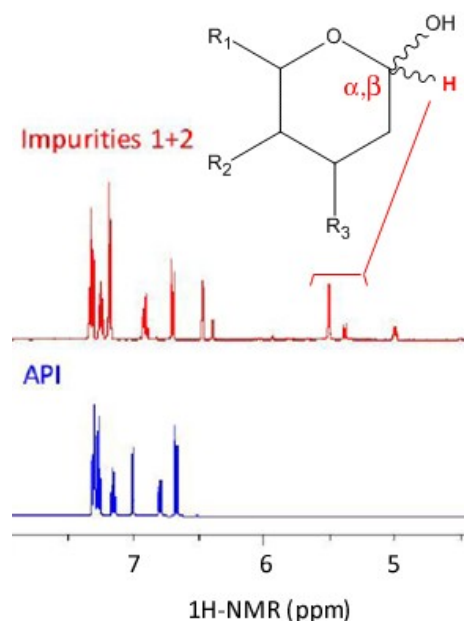


Figure 3. ^1H -NMR spectra of the API and of a mixture of the 2 API-related impurities. 2 new NMR resonances are observed in the region of hemi-acetalic function, relating to the 2 hemi-acetal forms.

Summary

The combination of LC-SPE-cryoNMR and LC-MS supported the **elucidation of this impurity problem in about 2 weeks while it remained unsolved at our client site for over 10 months**. This case study clearly illustrates the power of the used methods and know-how of Spinovation's scientists that can solve hitherto irresolvable impurity issues.

Spinovation's highly experienced team of specialists on average **reduces by a factor of 3 to 4 the typical timeframe to be allocated to a drug impurity profiling project.**