Monitoring Pharmaceutical Impurities

August 28, 2017 by Dr. Timothy Cross

In this previous article I interviewed Dr. Christian Zeine from LGC as to what pharmaceutical impurities are, why we analyse them and the regulations surrounding pharmaceutical impurities. In this article, Christian and I will look further into the analytical methodologies and workflows for the analysis, characterisation and monitoring of pharmaceutical impurities.

The workflow for the characterisation and ongoing monitoring of pharmaceutical impurities can be simply broken down into three main areas: separation, identification and quantification; but there are many different options available at each of these stages which I will outline below.

Analysis of Pharmaceutical Impurities

There are many different methods for the separation of pharmaceutical products in to the API (Active Pharmaceutical Ingredient) and associated impurities, such as electrophoresis and chromatography. High-Performance Liquid Chromatography (HPLC) is commonly used as is demonstrated in this review article. HPLC and UHPLC offer the ability for high resolution separation of a wide range of analytes with high-throughput and reproducibility. Alternative chromatographic methods to HPLC include Gas Chromatography (GC) for volatile organic compounds and Ion Chromatography (IC) for ionic analytes.

The chromatographic separation is typically hyphenated to detect and identify the analytes. HPLC is typically hyphenated to either UV-based detectors or mass spectrometry. With mass spectrometry, the API and impurities are detected and accurately identified by software. For more information on mass spectrometry technology please refer to this overview. The benefits of using mass spectrometry for impurity analysis are the accuracy and sensitivity it offers, lower limits of detection and the ability to be able to identify all the components, both the knowns and unknowns. Drawbacks include the additional cost of the mass spectrometer and also the perceived instrument complexity of operation and additional software packages. However, recent developments are overcoming these obstacles. For example, with the Thermo Scientific™ ISQ™ EC Single Quadrupole Mass Spectrometer, where the system is operated through open access Chromatography Data System (CDS) software (Thermo Scientific™ Chromeleon™ CDS Software), ensuring only one software is used for the complete workflow to reduce complexity. UV detection is frequently used for impurity detection as it is a simple, sensitive and robust method, especially hyphenated to HPLC. UV detection does not directly identify the compounds, but this can be performed using either MS or reference standards and then switching to UV and using the retention time to identify the compounds – as long as your HPLC system has outstanding retention time precision! More challenging is the analysis though in the early part of candidate development. Many hundreds or thousands of structures are synthesized but no reference standard material is available. For those situations and where no chromophores are present in the impurities, charged aerosol detection (CAD) has proven to be a great tool. With mass, structure and ionization independent response, the CAD delivers a much better, semi-quantitative impurity profile than UV/vis detection. The combination of single quadrupole mass spectrometry and CAD is one of the most powerful but easily routinely

Once you have identified the accurate mass and thus have a likely sum formula at hand, libraries can make suggestions on possible organic structures behind the peak. Assuming you are working on a special finished dosage form (FDF), for example the aspirin tablet from our first article you can narrow down those structure suggestions to anything that might have to do with the aspirin structure. That would comprise starting materials, intermediates, by-products and possible degradants. The peak can also stem from the interaction of any of those (or of the aspirin as the API) with excipients used to produce the FDF. Excipients can be binders, fillers, colourants or flavours, among others.

That short list then with possible suspects, how can you finally prove the final structure behind it? You might be able to narrow it down further by the study of dissociation patterns on further MS experiments, or to find some more hints in the literature. But the ultimate proof you can only make, at least once in the process of identifying an impurity, by the use of a reference standard of the same structure. You compare retention time, and if the same also UV and MS spectra of both standard and unknown peak for exact matches.

The impurity reference standard has to be carefully characterised itself for correct identity. You will find different qualities of standards or research materials on the market. Check the certificate of analysis beforehand if possible, to make sure it contains all data you would need to accept the identity of the standard, and in turn, your unknown peak.

Depending on the quality of the reference standard, it might also be suitable for quantitative work on the impurity in your API or FDF. LGC's webinar section provides information to see how good a characterisation of an impurity standard is good enough. In general, you should make sure that the reference you would use is not overestimated grossly for its purity respective assay content, because otherwise you would overestimate the impurity in your API or FDF as well. This can have negative consequences like possible OOS results or exceeding the ICH threshold for impurity qualification. OOS results can be costly. And the need to qualify an impurity (i.e. to show that is not harmful to the patient during the duration of use) can delay the time to market for a medicine considerably.

Workflow Enhancements

While the HPLC-UV/MS workflow for monitoring pharmaceutical impurities is well established, there are always ways to improve the process to make it more robust and also shorten the time to results — all leading to a more cost effective process. This application note on Nevirapine impurity profiling shows a good example of an enhanced workflow for pharmaceutical impurity analysis. In the application note, the latest UHPLC and column technology are utilised to reduce the separation time to 2.8 minutes compared to the 80 minute isocratic USP method and achieve sensitive detection over a wide dynamic range using a UV-based diode array detector (DAD).

Summary

There are multiple analytical options for analysing pharmaceutical impurities, however chromatographic separation by HPLC / UHPLC followed by either UV or MS detection and supported by the use of reference standards where necessary is the most common approach. Even with this established workflow there are options to improve to reduce complexity, throughput and ultimately cost such as shortening separation times, reducing instrument and software complexity and making them more user-friendly or using more cost-effective detection methods such as UV or charged aerosol detection. These improvements to workflows can be made whilst still complying with the regulations and guidelines provided by the regulatory bodies – should you be evaluating your current pharmaceutical impurities workflow to investigate whether you can enhance it and reduce costs?

Additional Resources

- Read more on achieving confident impurity detection with single quadrupole mass spectrometry in this application note
- Discover LGC's range of over 3500 impurity reference standards sorted by API here

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